EVALUATION OF SELECTED PROVENANCES OF *TAXODIUM DISTICHUM* FOR DROUGHT, ALKALINITY AND SALINITY TOLERANCE

A Dissertation

by

GEOFFREY CARLILE DENNY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Horticulture

EVALUATION OF SELECTED PROVENANCES OF *TAXODIUM DISTICHUM* FOR DROUGHT, ALKALINITY AND SALINITY TOLERANCE

A Dissertation

by

GEOFFREY CARLILE DENNY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	Michael A. Arnold
Committee Members,	Leonardo Lombardini
	Wayne A. Mackay
	W. Todd Watson
Head of Department,	Tim D. Davis

May 2007

Major Subject: Horticulture

ABSTRACT

Evaluation of Selected Provenances of *Taxodium distichum* for Drought, Alkalinity and Salinity Tolerance. (May 2007)

Geoffrey Carlile Denny, B.S., Texas A&M University;

M.A., The University of Texas

Chair of Advisory Committee: Dr. Michael A. Arnold

Taxodium distichum (L.) Rich. is a widely adaptable, long-lived tree species for landscape use. It is tolerant of substantial soil salt levels, but tends to defoliate in periods of extended or severe drought, when leaves come into contact with salty irrigation water, and tends to develop chlorosis on high pH soils. The purpose of this research was to identify provenances which may yield genotypes tolerant of these stresses. The appropriate name for baldcypress is *Taxodium distichum* (L.) Rich. var. *distichum*, for pondcypress is *T. distichum* var. *imbricarium* (Nutt.) Croom, and for Montezuma cypress is *T. distichum* var. *mexicanum* Gordon.

A germination study of *T. distichum* var. *mexicanum* revealed that if immediate germination of ripe seed is desired, then the best treatments are a citric acid soak and hot water baths, however, if seeds can be stratified, then no pre-germination seed treatment is needed. Citric acid scarification and hot water baths produced the best germination. Stratification hastened germination rates and cumulative mean germination percentages. Stratification for 45 d appears to be sufficient, although for the best pre-germination treatments stratification requirements were less pronounced. Greenhouse screening studies of open-pollinated families for drought tolerance show genotypes from eastern

localities were less tolerant than western genotypes. *Taxodium distichum* likely relies on both drought avoidance and drought tolerance strategies to deal with drought stress. A field screening for alkalinity tolerance showed a strong geographic component to the variation in tolerance of alkaline soils. When selecting plant material for an alkaline site, genotypes from Mexico and south Texas should be preferred, followed by central Texas genotypes. Greenhouse salinity screening showed that most genotypes tolerate moderate levels of soil salts, but at high soil salinities the tolerance appears to be highly genotype-dependent, rather than having a strong geographic pattern. Field evaluations demonstrated that *T. distichum* var. *mexicana* grew more rapidly on three Texas sites than the other varieties. These evaluations also suggest that when selecting plant material for an alkaline or xeric site, Mexican and south Texas genotypes should be preferred, followed by central Texas genotypes. Cold tolerance was not determined north of USDA hardiness zone 8.

ACKNOWLEDGEMENTS

I would like to acknowledge all the people who have helped me along the way. Thanks to: Mike Arnold for his guidance, advice, patience, and friendship; Wayne Mackay for his help and advice, and for not telling all the stories from El Paso; Leo Lombardini and Todd Watson for their support in getting me through this process; Dr. Brent Pemberton and Will Roberson for taking care of my field plots in Overton; and all the faculty, staff and students in the Department of Horticultural Sciences who helped with advice, supplies or labor.

Thanks to Mike Moore and "Peanut" Jackson for helping me make it through the Mexico trip. Thanks to Garry and Jay for helping to keep me relatively sane.

I owe special thanks to Lindsay Holmes for all her patience and support, as well as the pre-dawn harvests.

I owe a debt of gratitude to my parents, Jerry and Peggy Denny, and my little sister Erin for their unflagging support and love and for their financial backing. I hope they don't want interest!

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

LIST OF TABLES

TABLE Page

CHAPTER I

INTRODUCTION

Drought, salinity, and alkaline soils are common problems faced by many arborists, urban foresters, landscapers and homeowners. Kelsey and Hootman (1990) found that many urban street tree planter soils could be classified as saline or sodic, soil types that usually occur in arid or semi-arid areas with a higher evapotranspiration than precipitation. In many parts of the United States, drought and irrigation restrictions are becoming more common (Beeson et al., 2004).

Description of *Taxodium distichum*

Taxodium distichum (L.) Rich. is a widely adaptable tree species for landscape use, tolerating both wet and dry soils, and air pollution (Cox and Leslie, 1988; Wasowski and Wasowski, 1997). Watson (1983) reports tolerance to varying nutrient availability conditions, a wide range of soil aeration levels, and somewhat extreme pH levels. It is fast growing, has reliable feathery foliage, and a nice form (Arnold, 2002; Cox and Leslie, 1988). Two varieties, var. *distichum* (baldcypress) and var. *imbricarium* (Nutt.) Croom (pondcypress), have fairly good fall color some years, while var. *mexicana* Gordon (Montezuma cypress) remains semi-evergreen (Arnold, 2002). It is an extremely long-lived tree, with a life span of up to 700 years possible (Cox and Leslie, 1988). All of these factors allow *T. distichum* to tolerate many environmental stresses, making this a promising choice for urban landscapes. However, there are a few

---

This dissertation follows the style of The Journal of the American Society for Horticultural Science.

limitations to this species. While it is tolerant of substantial soil salt levels, it tends to defoliate when leaves come into contact with salty irrigation water, tends to develop chlorosis on sites with high pH, and has a tendency to "brown out" in periods of extended or severe drought (Arnold, 2002).

The Concept of Provenance in Landscape Horticulture

Arnold (2002) defines ecotypic variation as "a distinct morphological or physiological form, or population, resulting from (natural) selection by a distinct ecological condition". It is the entire basis for provenance studies (Arnold, 2002). Zobel and Talbert (1984) define a provenance as "the original geographic area from which seed or other propagules were obtained" and equate it to the concepts of geographic source and geographic race. They also present a more useful definition of the concept as "a subdivision of a species consisting of genetically similar individuals, related by common descent, and occupying a particular territory to which it has become adapted through natural selection" (Zobel and Talbert, 1984). Provenances can be determined by numerous characteristics of the geographical origin, including latitude, altitude, precipitation, temperature, soil, and day length (Zobel and Talbert, 1984). The identification of adapted provenances allows industry professionals to more closely tailor their plant selections to specific situations and can offer "the largest, cheapest and fastest gains" in tree improvement programs seeking an improved product for use in difficult ecophysiographic situations (Zobel and Talbert, 1984).

In forest management, the concept of seed source and provenance are widely recognized and used (Zobel and Talbert, 1984). However, in landscape horticulture and

urban forestry it is less widely utilized (Arnold, 2002). Some of the taxa studied in provenance research in this field include maple (*Acer* L.) spp. (Zwack et al., 1998; St. Hilaire and Graves, 2001), seaside alder (*Alnus maritima* (Marsh.) Muhl. ex Nutt.) (Schrader and Graves, 2000), pecan (*Carya illinoinensis* (Wangenh.) K. Koch) (Wood et al., 1998), Atlantic whitecedar (*Chamaecyparis thyoides* (L.) B.S.P.) (Jull et al., 1999), Amur maackia (*Maackia amurensis* Rupr.) (Pai and Graves, 1995), and sycamore (*Platanus occidentalis* L.) (Shoemake, 1996; Shoemake and Arnold, 1997).

Genetic variation in baldcypress has had some limited attention in past research. Beilman (1947), Flint (1974), McMillan (1974), and Sharma and Madsen (1978) all looked at seed source/provenance variation. Faulkner and Toliver (1983) found source effects for cone size and seed weight, but failed to find geographic variation for number of insect galls per cone, height, and diameter. However, they claimed that the "scope of this study was not large enough to detect geographic variation". Most of their seed sources were in Louisiana, Mississippi, and Arkansas, with only three seed sources outside of those states (two from Illinois and one from Texas). Pezeshki et al. (1995) found that there is a potential for differentiation among populations of baldcypress in salt tolerance. Krauss et al. (1996, 1998, 1999) also looked at intraspecific variation of salinity tolerance in baldcypress. All of the above-mentioned studies dealt with *Taxodium* as a forest tree, rather than an ornamental/urban tree. St. Hilaire (2001) reported that seed origin of *T. distichum* var. *mexicana* had no effect on cumulative germination percentage for two seed sources from New Mexico.

Drought Resistance

Water is one of the most important factors affecting plant growth and productivity (Taiz and Zeiger, 1998). Any decrease in the availability of water has implications for processes from solute transport to photosynthesis (Taiz and Zeiger, 1998). Pugnaire et al. (1999) define water stress as "the induction of turgor pressure below the maximal potential pressure…the magnitude of such stress is determined by the extent and duration of the deprivation". Plants regularly must withstand water deficits and have evolved physiological responses and ecological strategies that allow them to survive (Pugnaire et al., 1999). They can cope with water stress in two general ways: 1) drought avoidance and 2) drought tolerance (Levitt, 1980). Most plants generally adapt strategies somewhere along a continuum between these categories of mechanisms.

Drought avoidance strategies involve adaptations that minimize water loss, maximize the acquisition of available water, or the restriction of plant processes to periods of higher water availability (Levitt, 1980). These strategies may include changes in leaf morphology, increased stomatal sensitivity, changes in root characteristics, osmotic adjustment, changes to the cells' elastic properties and water storage, the use of alternative metabolic pathways (i.e. CAM and C_4 photosynthesis) and drought-induced dormancy (Pugnaire et al., 1999).

Changes in leaf characteristics are especially important in drought avoidance (Pugnaire et al., 1999). Plants can reduce water loss by increasing the diffusional resistance, reducing the evaporative area, and creating a buffer zone around the

evaporative surface (Levitt, 1980; Pugnaire et al., 1999). Increased diffusional resistance can be achieved by locating stomata in depressions or pores, and increasing cuticle thickness (Pugnaire et al., 1999). Species with superior drought resistance commonly have thicker cuticles compared to those of mesophytic taxa (Levitt, 1980). Plants can reduce their evaporative area in several ways. Leaves can get smaller and thicker (Pugnaire et al., 1999), or leaves can be rolled, folded or shed (Levitt, 1980). A buffer zone, which decreases water loss, can be created around the evaporational surface with pubescence (Pugnaire et al., 1999) or can be achieved with a compact foliage and crown form (Levitt, 1980).

Stomatal control is another way in which plants deal with drought stress (Levitt, 1980). The plant must balance the benefit of stomatal closure conserving water against limiting CO_2 uptake and decreased evaporative cooling of the leaf tissue (Lambers et al., 1998). Increased stomatal sensitivity is commonly observed in both xerophytes and mesophytes exposed to water deficits (Levitt, 1980). Levitt (1980) reports that there are two mechanisms of water stress-induced stomatal closure. The first is hydropassive stomatal closure, resulting from dehydration of the guard cells. This is not a very effective mechanism of water conservation because it usually requires relatively severe dehydration (Levitt, 1980). The second mechanism is hormonal control of stomatal aperture. An increase in abscisic acid (ABA) concentrations and/or decreased cytokinin concentrations are observed in water stressed plants, reducing stomatal aperture and inhibiting transpiration, while inducing a decline in leaf growth (Pugnaire et al., 1999). This mechanism allows water conservation by causing stomatal closure before leaves

become severely wilted and it keeps stomata closed during the reabsorption of water until it is safe to open them again (Levitt, 1980). The reduction in shoot growth allows for the accumulation of carbohydrates at a time when photosynthesis is declining (Pugnaire et al., 1999). Chaves et al. (2003) stated that ABA regulation of stomatal aperture is complex and "involves both long-distance transport and modulation of ABA concentration at the guard cell to a given dose of the hormone". They claimed that some of the factors that modulate stomatal response are the pH of xylem sap and that of leaf tissue. The pH of these tissues is affected by evaporative demand, light intensity and temperature (Chaves et al., 2003). Chaves et al. (2003) offer another possible mechanism for stomatal control. They stated that there is evidence that the rate of water supply may regulate stomatal changes. The supply rate can be affected by changes in xylem conductance or changes in leaf turgor (Chaves, 2003). This explanation supports the theory that plants utilize stomatal closure to prevent the loss of water transport capacity; the "stomata acting as pressure regulators that prevent xylem pressure from runaway cavitation thresholds" (Chaves et al., 2003).

Changes in root characteristics can facilitate drought avoidance in several ways (Levitt, 1980). The first is by decreasing water movement from roots into dry soil, which is accomplished by decreasing root surface and/or permeability to water (Levitt, 1980). Increased biomass allocation to root systems is the second change (Levitt, 1980). This allows the plant to explore a larger volume of the soil for extractable water. The third mechanism suggested is an increase in root resistance to water flow. This

mechanism relates back to the water supply theory of stomatal regulation discussed above.

Osmotic adjustment is a decrease in osmotic potential greater than can be explained by dehydration-driven solute concentration (Kramer, 1983). During osmotic adjustment, plants accumulate osmotically-active solutes, allowing turgor and turgor-related processes to continue in periods of water stress (Pugnaire et al., 1999). There are many solutes that can act as osmolytes, including inorganic ions, organic acids, sugars and amino acids, proline in particular (Pugnaire et al., 1999). Sugars and amino acids are commonly utilized because of the convenience of storing these osmolytes in large molecules like starch and protein, which are osmotically inactive, can serve other purposes in the cell and can be readily converted in times of stress (Pugnaire et al., 1999). Cyclitols (cyclic polyols) are often listed as plant stress metabolic products (Merchant and Adams, 2005). They have low molecular weights, are relatively inert at high concentrations, and are temporally stable. Merchant et al. (2006) found that in species of *Eucalyptus* L'Her. native to xeric regions, cyclitols were the major osmolytes utilized, while sucrose was the major osmolyte in species from mesic areas. They hypothesized that this represents contrasting physiological responses to water deficit. The xeric species utilize the more temporally stable molecules suggesting that this represents an acclimation to drought, where as the mesic species use sucrose which is easily metabolized and therefore beneficial in short-term water deficits. Cyclitols have been reported to have other "osmoprotective" roles in the cell, including cryoprotection, hydroxyl scavenging, and excess photochemical energy sequestration (Merchant and

Adams, 2005). Fructans reportedly have a secondary function of membrane stabilization; sorbitol, mannitol, and proline all serve as hydroxyl scavengers; and glycinebetaine has protective functions as well being osmolytic (Lambers et al., 1998). Kramer (1983) states that osmotic adjustment does not persist long after the removal of the stress, only occurs over a limited range of water potential, and does not fully maintain physiological processes.

Lambers et al. (1998) state that the extent to which cells can shrink in response to dehydration, and thereby the extent to which their water potential can decrease before the turgor loss point is reached, is dependant on their cell wall elasticity. Plants with more elastic cell walls can store more water and can lose more water before they reach their turgor loss point (Lambers et al., 1998). This allows plants with more elastic cells to maintain a lower osmotic potential and a higher turgor pressure at a given relative water content compared to plants with less elastic cells (Lambers et al., 1998). Water storage is important in succulents, but probably plays only a minor-role in long term dehydration postponement in mesophytes (Kramer, 1983). This is because daily water loss in periods of high evapotranspirational demand often exceeds the total water content of the plant, however if "stored water enables photosynthesis to continue for an additional hour each day it would be beneficial" (Kramer, 1983).

The use of alternative metabolic pathways (i.e., CAM and C_4 photosynthesis), ephemeral life cycles and drought-induced dormancy restrict plant processes to periods of higher water availability (Levitt, 1980). The number of taxa that utilize alternative

metabolic pathways is relatively limited, while ephemeral life cycles and drought-induced dormancy are characteristics that limit the usefulness of a plant in the landscape.

Drought tolerance mechanisms are generally more specific in the type of protections they provide compared to drought avoidance mechanisms (Levitt, 1980). These mechanisms can be divided into two general groups (Levitt, 1980). The first offers protection from direct strain or damage and the other from starvation.

Direct strain or damage includes the loss of proteins, nucleic acids, and cell membranes because of the accumulation of toxic ions and/or reactive oxygen species (ROS) (Levitt, 1980; Navari-Izzo and Rascio, 1999). As discussed above, many of the compounds synthesized during osmotic adjustment may protect the cell membrane and metabolic machinery under water deficit conditions (Chaves et al., 2003). These osmoprotective compounds include carbohydrates (e.g., fructans and sucrose), cyclitols and polyols (e.g., D-pinitol and mannitol), amino acids and hydrophilic proteins (e.g., proline, aspartic acid, glutamic acid, and late embryogenesis abundant (LEA) proteins), and methylated quaternary ammonium compounds (e.g., glycine betaine and alanine betaine) (Chaves et al., 2003). Chaves et al. (2003) stated that these solutes' protective function result from their interaction with water molecules rather than proteins. They help to stabilize membranes and protein complexes by sequestering water (Chaves et al., 2003). Chaves et al. (2003) also state that because these mechanisms are probably not functional until severe dehydration occurs, they are probably more critical to survival, rather than an increase in growth or yield. Cell membranes also adjust their lipid composition under water deficit conditions, which is believed to confer increased

tolerance (Navari-Izzo and Rascio, 1999). Antioxidant molecule and enzyme production and activity are increased during periods of water deficit (Chaves et al., 2003; Navari-Izzo and Rascio, 1999). These include superoxide dismutases (SODs), which help convert superoxide radicals to hydrogen peroxide, and catalases (CATs) and the components of the ascorbate-glutathione cycle which are responsible for the removal of H_2O_2 (Chaves et al., 2003; Navari-Izzo and Rascio, 1999).

Reduced photosynthesis occurs during water deficit because stomatal closure reduces the internal concentration of CO_2 available for fixation and because of metabolic inhibition and can lead to plant starvation (Chaves et al, 2003). The relative importance of these two factors depends on the rate of imposition and severity of the water deficit, plant phenology, plant taxa, and the superimposition of other stresses (Chaves et al., 2003). Plants can avoid this stress in several ways including increased water use efficiency (WUE) and stomatal opening at low water potentials (Levitt, 1980). Water use efficiency is the ratio between biomass produced and the amount of water lost (Lambers et al., 1998). Plants with a higher WUE need less water to produce the same amount of biomass as compared to plants with lower WUE. Levitt (1980) states that a moderate degree of drought tolerance may actually be due to the specific dehydration avoidance of guard cells, allowing the stomata to remain open.

Urban surfaces and compacted soils frequently decrease the amount of water that infiltrates into the root zone of trees, thus increasing competition with turf and other vegetation for the available water (Zwack and Graves, 1998). Therefore, water deficit situations can be common in urban areas. Zwack and Graves (1998) pointed out a need

for "tree taxa that maintain landscape function during episodes of variable and adverse soil moisture". St. Hilaire and Graves (2001) suggested that a strategy for selecting ornamentals with "superior resistance to drought stress" was to select from populations native to relatively xeric habitats. This is supported by Tuomela (1997), who found that provenances of *Eucalyptus microtheca* F.J. Muell. from dry regions exhibited "drought-resistance features…that may relate to prolonged annual drought in their natural habitats". Li (1998) found that *E. microtheca* seedlings from more arid provenances produced leaf dry mass/ turgid mass ratios, osmotic potentials at full turgor, and bulk moduli of elasticity favorable for drought resistance compared to mesic provenances when exposed to drought conditions. Li et al. (2000) found the same pattern. They found in *E. microtheca* a positive correlation between mean driest quarter rainfall of the site of origin and total biomass, height, transpiration, and specific leaf area (Li et al., 2000) of the provenance and a negative correlation between mean driest quarter rainfall and root mass/foliar area ratio, foliar area/stem cross sectional area, WUE, and $\delta^{13}C$ (Li et al., 2000). $\delta^{13}C$ is the carbon isotopic composition of a sample compared to a standard and is an indicator of the water use efficiency in plants (Lambers et al., 1998). Li et al. (2000) also reported that the provenances from dry areas had a larger foliage/stem area ratios, lower transpiration rates, and shorter hydraulic pathways. Cregg (1994) found that drought tolerance of *Pinus ponderosa* Dougl. ex Laws. can be improved through seed source selection. Cregg (1994) also reported that provenance differences in morphological and physiological traits related to drought resistance were noted in *Cercis canadensis* L., *Pinus taeda* L., *Pinus radiata* D. Don, *Juglans nigra* L.,

and *Psuedotsuga menziesii* (Mirb.) Franco. Abrams et al. (1990) found morphological and physiological traits related to drought resistance in provenances of *Fraxinus pennsylvanica* Marsh. from xeric areas, as did St. Hilaire and Graves (2001) in provenances of maple (*Acer saccharum* Marsh.). Griffin et al. (2004) found that xeric ecotypes of eastern redbud (*Cercis canadensis* L.) had higher instantaneous water use efficiencies than mesic ecotypes.

Salinity Tolerance

Thirty three percent of irrigated land worldwide is affected by salinity (Marschner, 1995). Saline soils are especially abundant in the arid and semiarid regions of the world, usually because the rainfall is insufficient for proper leaching (Marschner, 1995). However, salt may be added to soils by rain and sea spray in some regions, especially coastal areas, and by road salt in others. Irrigation water may have 100-1000 $g \cdot m^{-3}$ of salt, and with an average irrigation application of 10,000 $m^3 \cdot ha^{-1} \cdot year^{-1}$, 1-10 tons of salt can be added to the soil (Marschner, 1995). Sodium chloride is usually the dominant salt in problem areas, although other salts may be abundant as well, depending on the water source and the solubility of the salt (Marschner, 1995).

There are three ways that salinity restricts plant growth. First, it can induce water deficit. This is because the salts decrease the water potential of the rooting substrate which limits the ability of the plant to absorb water (Marschner, 1995; Wahome et al., 2001). Second, the plant can experience ion toxicity because of excess uptake of certain ions, most commonly Na^+ and Cl^- (Marschner, 1995; Wahome et al., 2001). Lastly, salt stress can cause an ion/nutrient imbalance in the plant. This is

usually due to a decrease in the plants' ability to absorb certain nutrients and to transport nutrients internally (Marschner, 1995; Wahome et al., 2001), often as a result of competition with Na^+ or other cations.

Wahome et al. (2001) define salt tolerance as "the ability of a plant to maintain growth and metabolism under saline conditions". There are two mechanisms that a plant can use to adapt to a saline substrate: salt exclusion and salt inclusion (Marschner, 1995). Salt exclusion is by far the more common strategy of salinity tolerance among non-halophytes, and requires the plant to be able to avoid internal water deficit (Marschner, 1995). The plant's ability to prevent translocation of ions, especially Na^+, from the roots to the shoots is very important for excluders, because the exclusion of ions from the cell is not usually sufficient (Wahome et al., 2001). Inclusion usually requires a tolerance of high levels of ions, especially Na^+ and Cl^-, in the cytoplasm (Marschner, 1995), but may also involve sequestration of ions in cellular compartments. This strategy is normally limited to halophytes. The ionic component of salt stress is met with two major strategies by plants, salt exclusion and salt inclusion/compartmentation (Jacoby, 1999). Plants do not fall into one category or the other, but rather somewhere along a continuum between the two (Marschner, 1995). Salt inclusion as a strategy usually involves the utilization of the salt ions for osmoregulation and the replacement of K^+ in several metabolic functions by Na^+. Inclusion of ions is generally found in halophytes (Jacoby, 1999).

Classification of salt tolerance in plants is commonly based on a threshold electrical conductivity (EC) above which plant growth and development is impaired and

on the rate of the decrease in growth after the threshold EC has been reached (Marschner, 1995). Large differences in intraspecific salt tolerance have been observed, as well as differences between ontogenetic stages of individuals (Marschner, 1995).

Plant strategies for tolerance of the osmotic portion of are basically the same as those for drought tolerance, i.e. osmotic adjustment, reduced transpiration, changes in cell wall elasticity, and decreased respiration rates (Jacoby, 1999). All of these adaptations are discussed in detail above.

Typically, glycophytes rely more heavily on the exclusion strategy (Gucci et al., 1997) and halophytes tend to utilize the salt inclusion strategy (Jacoby, 1999). Salt exclusion by plants is achieved in several different ways. The first is to limit the uptake of the salt ions. This is achieved by increased selectivity of trans-membrane transporter proteins, especially the K^+/Na^+ selectivity of the cation channel (Amtmann and Sanders, 1999), and decreased membrane permeability for certain ions, such as Cl^- (Lambers et al., 1998). Membrane selectivity is a critical step because solutes are generally required to enter the symplastic pathway to enter the cortex of the root and the vascular tissue because of the Casparian band in the endodermis of roots (Marschner, 1995). However, not all of the salt ions can be excluded in this way and for this reason plants rely on extrusion and sequestration (Jacoby, 1999).

Extrusion can take the place of active efflux of excess salt ions from cells (Marschner, 1995) or the utilization of salt glands (Gucci et al., 1997). Salt glands are a specialized structure that secrete salts, usually from the leaves, and are not common among most plants (Gucci et al., 1997). Cellular extrusion takes the form of active

transport of ions out of the cell against a concentration gradient (Marschner, 1995). On of the most well understood transporters is the Na^+-H^+ antiporter which utilizes a proton gradient that is set up by an H^+-ATPase that utilizes ATP as an energy source to pump protons to the apoplast (Marschner, 1995). The Na^+-H^+ antiporter uses the energy of one proton moving in to expel one sodium ion (Marschner, 1995).

Plants also utilize sequestration to exclude salt ions from the cytosol (Jacoby, 1999; Marschner, 1995). This is generally achieved by concentrating the ions in the vacuole (Jacoby, 1999; Marschner, 1995). Plants usually increase the number of Na^+-H^+ antiporters in the tonoplast membrane, like the plasma membrane, when exposed to salinity (Jacoby, 1999; Marschner, 1995). These tonoplast antiporters also utilize a proton gradient set up by H^+ ATPases (Marschner, 1995). Salt ions that are translocated to shoots are often loaded into the phloem and retranslocated to the roots to be extruded or sequestered (Jacoby, 1999; Marschner, 1995). Some plants also partition salt ions to certain parts of the shoot, like older leaves, petioles, etc. (Marschner, 1995). This helps to protect the function of the younger and more productive tissues.

Plants are often more sensitive to foliar exposure to salts than substrate salts (Devitt et al., 2003). Surface properties of the leaf play a larger role in determining the sensitivity of the plant of foliar exposure to salts than the general salt tolerance of the species (Marschner, 1995). The cuticle of the leaf plays a similar role to the Casparian band in the root endodermis, preventing the direct entry of solutes into the shoot tissue (Marschner, 1995). The thickness of the cuticle plays a role in the sensitivity of plants to foliar salts. Generally, the thicker the cuticle, the lower the sensitivity to foliar salts

(Marschner, 1995). Also, there are hydrophilic pores through the cuticle that affect the sensitivity to foliar salts. The number, distribution and diameter of these pores all influence the size of the effect on the foliar salt sensitivity (Marschner, 1995). The interior of the pores is typically negatively charged, so cations pass through them more readily than anions (Marschner, 1995).

There is substantial information on variation in salt tolerance of *Taxodium*. Pezeshki et al. (1988) found that seedlings exposed to increasing levels of NaCl showed an increase in foliar Na, K, Ca, and Mg concentrations and a decrease in net photosynthesis and stomatal conductance. However, leaf internal CO_2 concentrations remained constant over the range of NaCl treatments, indicating that the observed decrease in net photosynthesis is due to the accumulation of excess ions rather than stomatal closure (Pezeshki et al., 1988). Allen et al. (1994) compared the performance of 15 open-pollinated families under increasing salinity levels. They found that families from brackish sources had greater total biomass, leaf area, and tolerance index values than freshwater sources. Allen et al. (1994) noted a broad range of seedling responses to salinity and suggested that "substantial gains in salt tolerance may be possible in the short term by simply using seed from brackish locations to produce seedlings". Pezeshki et al. (1995) also compared seedlings from a freshwater source to seedlings from a brackish site. Their findings are the reverse of Allen et al. (1994), the freshwater seedlings had higher growth rates and greater net photosynthetic capacity per unit leaf area (Pezeshki et al., 1995). While their data did not support the idea that seedlings from brackish sources "have a capacity to survive and grow better in saltwater", they claimed

that there is variation among populations for salt tolerance. Allen et al. (1996) noted that more tolerant families gradually lost older leaves and retained or produced new leaves, and salt sensitive families had tip dieback and refoliation basally. They stated that salt tolerance in *Taxodium* is probably due to relative ability to "exclude ions or effectively compartmentalize them in cell vacuoles", citing a higher concentration of Na and Cl ions in the leaves of less tolerant genotypes (Allen et al., 1996). Allen et al. (1996) also noted that at higher salinity levels there was a decrease in biomass allocation to root systems and an increase in root Na^+/Ca^{2+} and Na^+/K^+ ratios, indicating a breakdown in root function and membrane integrity. Krauss et al. (1998) found that the differential germination under saline conditions of seed from different sources of *Taxodium* was useful in screening for salt tolerance. Krauss et al. (1999) stated that screening for salt tolerance is best accomplished by seed germination trials, above-ground growth evaluation, gas exchange parameters, or ion uptake in root and shoot tissue. Conner and Inabinette (2005) reported substantial differences in survival and growth of seedlings from eight estuaries in the southeastern U.S. when subjected to saline conditions. Seedlings from sites in Louisiana, Alabama, and Florida were the best performers (Conner and Inabinette, 2005). They cited the need for a more detailed analysis of material from these areas to determine if they "represent sources of baldcypress seed possessing greater tolerance to saline conditions" (Conner and Inabinette, 2005).

Alkalinity Tolerance

The nutrients which are most limiting to plant growth on alkaline sites are Fe, P, Zn, and Mn (Marschner, 1995). The alkalinity tolerance in plants is often determined by

the ability of the roots to extract these nutrients, their nutrient uptake efficiency (Lambers et al., 1998; Marschner, 1995). There are both non-specific and specific mechanisms for the uptake of these nutrients (Marschner, 1995). The first non-specific mechanism is a root-induced decrease in the rhizosphere pH because of preferential cation uptake (Marschner, 1995). The second mechanism is the release of organic acids into the rhizosphere to decrease the pH (Marschner, 1995). The third non-specific mechanism is the release of photosynthates which support microorganisms in the rhizosphere (Marschner, 1995). These microorganisms alter the pH and the redox state of the soil and may release chelators, like siderophores, which the plant is able to take up (Marschner, 1995). Iron uptake in alkaline substrates is perhaps the best understood and has the most mechanisms identified (Marschner, 1995). Specific mechanisms for iron uptake include an increase in membrane-bound reductases, increased proton excretion, and a decreased sensitivity to iron transporters to high bicarbonate concentrations (Marschner, 1995; Valdez-Aguilar and Reed, 2006). The known mechanisms for phosphorus uptake are all in the non-specific category, while those for Zn and Mn are poorly understood (Marschner, 1995).

The other two general categories of alkalinity tolerance adaptive strategies are less well understood (Marschner, 1995). The second is a decreased sensitivity with little or no growth inhibition at high bicarbonate concentrations (Lambers et al., 1998; Marschner, 1995). The third category is the avoidance and tolerance of high calcium concentrations (Marschner, 1995). Both strategies probably involve the accumulations of the ions in the vacuole and the restriction of their uptake (Marschner, 1995).

In trees, and many other plants, ectomycorrhizal associations are important in alkalinity tolerance (Lambers et al., 1998). They release siderophores, increasing iron acquisition (Lambers et al., 1998; Marschner, 1995). Additionally, they may excrete oxalic acid, which dissolves calcium phosphate, facilitating phosphorus uptake and precipitating calcium oxalate, with the added bonus of decreasing calcium uptake (Lambers et al., 1998; Marschner, 1995).

There is evidence for variation in alkalinity tolerance among genotypes of woody plants. Ben-Ya'acov and Michelson (1995) report racial differences among *Persea americana* Mill. rootstocks in resistance to "lime-induced chlorosis" and cite limited or no information on the mechanisms behind the observed differences. Shi and Byrne (1995) report on variation in bicarbonate tolerance of *Prunus* L. rootstocks. Valdez-Aguilar and Reed (2006) found differential alkalinity tolerance in two cultivars of *Hibiscus rosa-sinensis* L. Wood et al. (1998) cite provenance differences in Zn deficiency in *Carya illinoinensis* (Wangenh.) K. Koch, as do Marcar et al. (2002) in provenances of *Eucalyptus camaldulensis* Dehnh. No information on alkalinity tolerance of *Taxodium* was found.

CHAPTER II

TAXONOMY AND NOMENCLATURE OF BALDCYPRESS, PONDCYPRESS AND MONTEZUMA CYPRESS

The appropriate taxonomy and nomenclature for plant taxa (taxonomic groupings) are often overlooked by the nursery industry and may seem of little importance. However, names frequently do not reflect the most current taxonomic classification. This may seem of little importance. However, names take on a new importance when we realize that binomials are intended to convey information beyond distinguishing one plant from another. Binomials reflect relationships and relatedness among groups of plants. We ignore that information if we do not use the most appropriate name as decided by a taxonomic specialist. Ecologically and environmentally friendly industry practices are growing in importance along with public interest. Issues such as provenance are receiving more and more interest and research (Bresnan et al., 1996; Giridhar et al., 1995; Lickey et al., 2002; McMillian, 1974; Schrader and Graves, 2000; Shoemake et al., 2004; Wood et al., 1998). Appropriate classification of taxa is of the utmost importance in these types of studies, because they attempt to investigate the geographic component to genotypic variation. Erroneous conclusions may be drawn if incorrect taxonomic classification is used.

Often horticulturists are frustrated, irritated and sometimes baffled by the seemingly arbitrary nomenclatural changes taxonomists and systematists make to plants. We often wonder aloud, “why can’t they just leave things alone?” We often ask students

the importance of scientific names while teaching plant materials classes. Answers range from "because plants can have more than one common name" to "so each plant has a standard name that everyone uses." Almost invariably the answers deal with distinguishing plants from each other. We spend very little time on the importance of scientific names for conveying relatedness among plants or evolutionary history of taxa. Yet, this is the objective of the hierarchical system Linnaeus created. Otherwise, all that would matter is that each taxon has a unique name. Name changes are not always meaningful if all we are concerned with is distinguishing one plant from another. However, as interest grows for issues like seed source, provenance, genetic pollution and threatened and endangered plant species, so does the need for an understanding of the relatedness and evolutionary history of plants. Correct plant nomenclature becomes more important as to which genus a species belongs to or whether two taxa are distinct species or subspecies or botanical varieties. Names must adhere to the nomenclatural rules set by the International Code of Botanic Nomenclature (Greuter et al., 2000) including those dealing with status, typification, and priority of names.

There are several very useful online nomenclatural data bases that are readily accessible. These include The International Plant Names Index (The International Plant Names Index, 2006), the Missouri Botanical Garden's VAST (VAScular Tropicos) nomenclatural database accessed through the W^3TROPICOS interface (Missouri Botanical Garden, 2006), and the USDA National Plant Germplasm System (United States Department of Agriculture – Agricultural Research Service, 2006).

Taxonomy of *Taxodium*

Baldcypress, pondcypress and montezuma cypress are three taxa recognized historically in the genus *Taxodium*. Baldcypress has a range along the Atlantic and Gulf Coast Plains of the United States. It can be found naturally as far north as Maryland and Illinois, south into Florida and central Texas (Middleton and McKee, 2004). Pondcypress is restricted in distribution to Florida and the Gulf Coast east of Texas, and up the Atlantic coast to North Carolina (Middleton and McKee, 2004). Montezuma cypress is the southern most taxa, occurring in Mexico and far south Texas (Turner and Watson, 1999).

T. distichum is widely accepted as the correct name for baldcypress (Arnold, 2002; Liberty Hyde Bailey Hortorium, 1976; Lickey and Walker, 2002; Lickey et al., 2002; McMillian, 1974; Murphy and Stanley, 1975; Schopmeyer, 1974; Tsumura et al., 1999; Turner and Watson, 1999; Vines, 1960; Watson, 1983; Watson, 1993). Disagreement arises in the classification of the other two taxa. Authors treat the genus as having three species (Arnold, 2002; Griffiths, 1994; Krüssmann, 1985; Turner and Watson, 1999) others as two species, one having two botanical varieties (Liberty Hyde Bailey Hortorium, 1976; Vines, 1960). Pondcypress has been treated as a distinct species, *Taxodium ascendens* Brongn., by some authors (Arnold, 2002; Griffiths, 1994; Krüssmann, 1985; Turner and Watson, 1999) and as a botanical variety of *T. distichum* by others (Liberty Hyde Bailey Hortorium, 1976; Lickey and Walker, 2002; Lickey et al., 2002; Murphy and Stanley, 1975; Schopmeyer, 1974; St. Hilaire, 2001; Vines, 1960; Watson, 1983; Watson, 1993). The varietal name most commonly assigned is *T.*

distichum var. *nutans* (Ait.) Sweet. Pondcypress has been segregated from baldcypress based on vegetative morphology, sometimes in combination with poorly defined and inconsistent habitat or community-type differences (Watson, 1983). Watson (1983) reported that there exist numerous individuals and populations that "exhibit morphological intermediacy or curious admixtures of the characters representative of the morphological extremes". This phenomenon has also been personally observed by the authors. Watson (1983) contends that the vegetative and ecological differences are insufficient to support specific rank for pondcypress. Also, there is no significant difference in reproductive morphology and anatomy, embryology, leaf flavonoid and biflavonoid profiles and nuclear DNA content of meristematic root cells (Watson, 1983). All these characters have been reliable criteria for distinguishing between species of other conifers (Watson, 1983). Therefore, Watson (1983) classified pondcypress as a botanical variety of *T. distichum.* Also, Tsumura et al. (1999) concluded that pondcypress should only be given varietal status based on DNA analysis using cleaved amplified polymorphic sequences. Based on allozyme analysis, varietal status for pondcypress was also supported by Lickey and Walker (2002).

Montezuma cypress is often treated as a distinct species, *Taxodium mucronatum* Tenore (Arnold, 2002; Liberty Hyde Bailey Hortorium, 1976; Creech and Yunlong, 2003; Griffiths, 1994; Krüssmann, 1985; McMillian, 1974; St. Hilaire, 2001; Turner and Watson, 1999; Vines, 1960). However, according to Watson (1983) it has also been treated as a botanical variety, *T. distichum* var. *mexicanum* Gordon. DNA and phytochemical analyses are not available for montezuma cypress. But, there are several

factors that suggest varietal status is appropriate. Montezuma cypress differs only by 'minor phenological characters', as indicated by Watson (1993) in his treatment of *Taxodium* for the Flora of North America. McMillan (1974) indicated morphological and physiological gradients exist between *T. distichum* and the Mexican populations. Montezuma cypress has been planted in close proximity in areas of central Texas to native baldcypress stands. Large numbers of offspring with intermediate characteristics and heavy seed set were observed there in the fall of 2003 (G.C. Denny, personal observation). These observations, in the absence of molecular evidence, indicate that varietal status is more appropriate for montezuma cypress. See Table 1 for a key to the botanical varieties of *Taxodium distichum*.

Nomenclature

Once the taxonomic questions are answered, the question of appropriate nomenclature arises. Baldcypress was originally described by Linnaeus (1753) as *Cupressus disticha*. Richard (1810) transferred to the genus *Taxodium* as *T. distichum*.

The correct botanical name for baldcypress is *T. distichum* var. *distichum* when the genus is treated as one species with three botanical varieties.

Pondcypress is commonly cited as *T. distichum* var. *nutans* (Ait.) Sweet, when it is given varietal status (Liberty Hyde Bailey Hortorium, 1976; Murphy and Stanley, 1975; Schopmeyer, 1974; St. Hillaire, 2001; Vines, 1960). According to Watson (1983), *Cupressus disticha* (L.) var. *nutans* was originally described by Aiton (1789) and *Cupressus disticha* var. *imbricaria* by Nuttall (1818). It appears *nutans* has priority. However, the plant described by Aiton (1789) was not pondcypress, rather it was a long

leaved, drooping form of baldcypress (Watson, 1983). Therefore, *imbricaria* has priority (Watson, 1983). The name *imbricaria* became *imbricarium* in 1837 when Croom (1837) classified pondcypress as a botanical variety of *T. distichum* (Watson, 1983). The correct botanical name for pondcypress is *T. distichum* var. *imbricarium* (Nutt.) Croom adopted by modern authors (Lickey and Walker, 2002; Lickey et al., 2002; Tsumura et al., 1999; Watson, 1983; Watson, 1993).

Nomenclature of montezuma cypress is much less complicated. When it is treated as a distinct species, the name is *Taxodium mucronatum* Tenore (Arnold, 2002; Liberty Hyde Bailey Hortorium, 1976; Creech and Yunlong, 2003; McMillian, 1974; St. Hilaire, 2001; Turner and Watson, 1999; Vines, 1960). However, because the most appropriate taxonomic treatment is as a botanical variety, the correct botanical name is *T. distichum* var. *mexicanum* Gordon (Watson, 1983).

In summary, we suggest the following classification of *Taxodium*: baldcypress *Taxodium distichum* var. *distichum*, pondcypress *Taxodium distichum* var. *imbricarium*, montezuma cypress *Taxodium distichum* var. *mexicanum*. See Table 2 for an abbreviated list of synonymy of the three taxa.

This chapter has been accepted in a revised form for publication in HortTechnology (Denny and Arnold, 2007a).

Table 1. Key to botanical varieties of *Taxodium distichum*. Adapted from Watson (1983) and Correll and Johnston (1970).[1]

1a) Determinate short shoots mostly ascending in a vertical plane; awl-like leaves narrowly lanceolate, 0.1-0.4 in long, appressed and imbricate in 5-8 ranks on shoots……..……..…… var. *imbricarium*

1b) Determinate short shoots mostly spreading in a horizontal plane; flattened leaves narrowly linear, 0.2-0.6 in long, divergent and appearing two-ranked on shoots…….……. 2

2a) Leaves deciduous; branches (catkins) containing male cones short and crowded, often divided into compact secondary branches……..… var. *distichum*

2b) Leaves semi-evergreen; branches (catkins) containing male cones long and slender, open, made up of single cones or clusters of several cones……...……..… var. *mexicanum*

---

[1]1 mm=0.0394 in

Table 2. Abbreviated synonymy for baldcypress, pondcypress and montezuma cypress. Adapted from Watson (1983) and Missouri Botanical Garden (2006).

Baldcypress

1810. *Taxodium distichum* (Linneaus) Richard var. *distichum*
- 1810. *Taxodium distichum* (Linneaus) Richard
- 1753. *Cupressus disticha* Linneaus
- 1789. *Cupressus disticha* var. *nutans* Aiton
- 1827. *Taxodium distichum* var. *nutans* (Aiton) Sweet
- 1938. *Taxodium distichum* f. *confusum* Palmer and Steyermark
- 1939. *Taxodium distichum* f. *pendens* Rehder

Pondcypress

1837. *Taxodium distichum* var. *imbricarium* (Nuttall) H.B. Croom
- 1818. *Cupressus disticha* Linneaus var. *imbricaria* Nuttall
- 1833. *Taxodium ascendens* Brongniart
- 1902. *Taxodium imbricarium* (Nuttall) R.M. Harper
- 1927. *Taxodium ascendens* var. *nutans* (Aiton) Rehder, pro synonym, non Aiton

Taxodium ascendens var. *nutans* (Aiton) Sweet, sensu authors, non Aiton.
- 1982. *Taxodium ascendens* ssp. *nutans* (Aiton) E. Murray, pro synonym, non Aiton.

Montezuma Cypress

1858. *Taxodium distichum* var. *mexicanum* (Carrière) Gordon
- 1855. *Taxodium mexicanum* Carrière
- 1853. *Taxodium mucronatum* Tenore
- 1854. *Taxodium montezumae* Decaisne
- 1906. *Taxodium distichum* var. *mucronatum* (Tenore) A. Henry

---

CHAPTER III

PRE-GERMINATION SEED TREATMENTS AND STRATIFICATION AFFECT GERMINATION OF MONTEZUMA CYPRESS

Taxodium distichum var. *mexicanum* (syn. *T. mucronatum*), montezuma cypress, is an adaptable tree species that is fast growing and has potential for large urban sites (Arnold, 2002, St. Hilaire, 2001). The cultural requirements for the other varieties in this species (baldcypress and pondcypress) have been established, but information on Montezuma cypress is lacking (Murphy and Stanley, 1975; Schopmeyer, 1974; St. Hilaire, 2001). Seed propagation is the most common form of propagation (St. Hilaire, 2001). Baldcypress seed germination is reported to be generally poor, with only 10% germination of control seeds after 60 d; however, various seed treatment combinations increase these percentages (Murphy and Stanley, 1975). The current study was undertaken to investigate the effects of the recommended seed treatment combinations for baldcypress and pondcypress on montezuma cypress and to determine if treatments were needed or useful. This study provides information on the germination requirements of montezuma cypress. This is important information for nursery growers because there is little known about the cultural requirements of this taxon of increasing importance in the landscape and nursery trade.

Taxodium distichum var. *mexicanum*, has a distribution from the lower Rio Grande Valley of Texas south into southern Mexico (Arnold, 2002; Correll and Johnston, 1970; Turner et al., 2003). It is a medium to large tree, usually less than 15.3 m (50 ft) tall in Texas, but the famous large tree of Santa Maria de Tule in Oaxaca,

Mexico, is estimated to be 38.1 m (125 ft) tall with a circumference of 50 m (162 ft) (Arnold, 2002; Correll and Johnston, 1970). Montezuma cypress is typically a seed-propagated species, but the germination requirements are not well known (St. Hilaire, 2001). St. Hilaire (2001) has conducted some studies to illuminate these requirements and found that mechanical scarification enhanced germination rates. Germination of both baldcypress and pondcypress has been studied and the requirements have been established (Murphy and Stanley, 1975; Schopmeyer, 1974; St. Hilaire, 2001). Murphy and Stanley (1975) recommend a 4 h soak in sulfuric acid to hasten germination of baldcypress and pondcypress. They found that warm-water soaks and cold stratification were no more effective than the untreated control, but had the advantage of producing a more uniform population of seedlings. They reported no embryo dormancy in baldcypress or pondcypress and low germination rates to be the result of a hard, impermeable seed coat. A 5 min soak in ethanol followed by 90 d cold stratification or soaking seeds in 3.3 °C (38°F) water for 90 d have also been reported as appropriate treatments to increase germination of baldcypress (Schopmeyer, 1974). A 24 to 48 h soak in 100 $mg \cdot L^{-1}$ citric acid followed by 60 to 90 d cold stratification was reported to enhance pondcypress germination (Schopmeyer, 1974).

The objectives of this study were 1) to determine the effect of previously recommended seed treatments for baldcypress or pondcypress on montezuma cypress seed germination, and 2) to determine the effects of stratification in combination with the above seed treatments on germination of montezuma cypress seeds.

Materials and Methods

Seeds of *T. distichum* var. *mexicanum* were collected from a single tree on 19 Sept. 2003, in Southmost, Texas (25°52′35′ N, 97°27′5′ W, elevation 4.5 m, U.S. Dept. Agr. Plant Hardiness Zone 9b). Cones were slightly immature and were allowed to dry at room temperature (22 °C, 71.6 °F) until treatments were applied. Seeds were separated from cone fragments by hand. Seven pre-germination treatments and three stratification periods were applied to the seeds starting on 4 Dec. 2003. Stratification took place in moist peat moss at 2 °C (35.5 °F) for periods of 0, 45 and 90 d. The seven pre-germination treatments were a 5 min ethanol soak (99.5% solution, Sigma-Aldrich, Inc., St. Louis, Mo.), a 5 min ethyl ether soak (99.5% solution, Sigma-Aldrich, Inc., St. Louis, Mo.), five hot water baths (42 °C, 107.6 °F) allowing the water to cool to room temperature between baths, a 48 h soak in 100 $mg \cdot L^{-1}$ (100 ppm) citric acid (EM Science, Gibbstown, N.J.), nicking the testa with a razor, stratification in water, and a non-treated control. For the 0 d stratification period the water stratification was carried out by placing the seeds in 500 ml of 22 °C (71.6 °F) water and then removing them immediately, for the 45 d and 90 d stratification periods, seeds were left in 22 °C (71.6 °F) water for their respective period. Germination was carried out in petri dishes (diameter = 9 cm (3.5 in)) with a double layer of moist filter paper in a growth chamber with 12 h day/night photoperiods and at a constant 25 °C (77 °F). Each dish contained 30 seeds. Three dishes per treatment combination randomly arranged within the growth chamber were evaluated (30 seeds per petri dish per pre-germination treatment per stratification time). Water (5 ml (0.17 oz)) was added to the petri dishes as needed to

keep the filter paper wet. The number of seeds germinated was counted daily for the first 14 d and then at 21 d. A seed was counted as germinated when the radicle protruded at least 2 mm (0.08 in).

Data were transformed with an arcsin transformation. Data were analyzed using univariate analysis (Table 3) in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.) and means were separated using Duncan's Mean Separation ($P \leq 0.05$).

Results and Discussion

Pre-germination treatment and stratification had significant effects on the germination rate of *Taxodium distichum* var. *mexicanum* (Table 3). Overall germination was low, with a maximum mean final germination percentage of only 32% (Table 4). This is consistent with Murphy and Stanley's (1975) reports on baldcypress and pondcypress germination.

Stratification and pre-germination treatment effects were confined to early stages of germination. All statistical differences ($P \leq 0.05$) among stratification and pre-germination treatments were manifested by seven days of germination time (Table 4), but were not apparent with longer (14 d or 21 d) germination times. Stratification treatments hastened germination, as expressed in increased mean germination percentages for 45 d and 90 d treatments germinating at 7 d (Table 4). The 90 d stratification treatments with ethanol, ethyl ether, citric acid and the control pre-germination treatments typically achieved their final germination rates sooner, than with 45 d or 0 d stratification treatments (Table 4). Stratification for 45 d usually produced the greatest mean final germination percentages, followed by 0 d and then 90 d

stratification, but variation occurred among pre-germination treatments (Table 4). Stratification for 90 d resulted in some seeds becoming soft and rotten, suggesting that the peak stratification time had been surpassed.

Pre-germination treatment also had an effect on germination rate, but only during the first seven days of germination. During the first 7 days of germination, citric acid treatment produced the most uniform germination and highest mean germination percentages, but not thereafter (Table 4). Aesthetically, the citric acid treatment produced the most robust seedlings compared to other pre-germination treatments. Seedlings produced appeared to be larger with darker green color compared to other treatments.

A 48 h soak in 100 $mg \cdot L^{-1}$ (100 ppm) citric acid appears to be the best pre-germination treatment studied, if stratification is not applied. Stratification tends to hasten germination. Stratification for 45 d is sufficient, with no improvement in cumulative germination at 90 d stratification. Although stratification hastened germination, this may not have been due to a physiological dormancy (endodormancy), but rather a physical one (ectodormancy). The stratification under moist conditions may simply have allowed the seed to imbibe sufficiently and thereby hasten germination when favorable growing conditions occurred. This would be in agreement with St. Hilaire (2001) and Murphy and Stanley (1975) who both suggest that there are no physiological dormancy requirements for germination. St. Hilaire (2001) found that removing the seed coats had a similar effect to stratification, hastening germination and improving germination uniformity. If immediate germination of ripe seed is desired,

then the best treatments are the citric acid soak and the hot water baths, however, if seeds can be stratified, then no pre-germination seed treatment is needed.

This chapter has been accepted in a revised form for publication in the Journal of Environmental Horticulture (Denny and Arnold, 2007b).

Table 3. ANOVA tests of between-subjects effects for seed germination of Montezuma cypress, *Taxodium distichum* var. *mexicanum*.

Source	Sum of Squares	df	Mean Square	F value	Significance ($P \leq$)
Corrected Model[z]	10,094.726	314	32.149	7.331	0.000
Intercept	12,146.607	1	12,146.607	2,769.919	0.000
Pre-germination Treatment (PT)[y]	636.815	6	106.136	24.203	0.000
Stratification (S)[x]	439.501	2	219.750	50.112	0.000
Day (D)[w]	7,271.202	14	519.372	118.438	0.000
PT * S	362.277	12	30.190	6.884	0.000
PT * D	375.598	84	4.471	1.020	0.436
S * D	673.928	28	24.069	5.489	0.000
PT * S * D	335.405	168	1.996	0.455	1.000
Error	2,762.667	630	4.385		
Total	25,004.000	945			
Corrected Total	12,857.393	944			

[w]Germination percentages determined at 7, 14, and 21 days.
[x]Stratification at 2 °C (35.5 °F) in moist peat moss for 0, 45,and 90 d.
[y]The seven pre-germination treatments included ethanol, ethyl ether, hot water, water, citric acid, mechanical, and control.
[z]R^2 = .785 (Adjusted R^2 = .678; Adjusted R^2 is an R^2 adjusted for the number of terms in a model and only increases if the terms added to the model improves it more than would be expected by chance. Adjusted R^2 can be negative and will always be lower than R^2. Adjusted R^2 = 1 - [(SS(error)/DF(error)) / (SS(total)/DF(total))])

Table 4. Mean daily germination percentages for pre-germination treatments of montezuma cypress, *Taxodium distichum* var. *mexicanum*.

		Stratification period (days)		
		0[z]	45	90
Day	Seed treatment	Percent germination		
7[y]	Ethanol	0.0 Bb[x]	4.4 Ba	17.8 Aa
	Ethyl ether	0.0 Bb	1.1 Ba	18.9 Aa
	Hot water	12.2 Aa	2.2 Ba	14.4 Aa
	Water	0.0 Ab	5.6 Aa	11.1 Aa
	Citric acid	15.6 Ba	11.1 Ba	28.9 Aa
	Mechanical	0.0 Ab	8.9 Aa	14.4 Aa
	Control	0.0 Bb	2.2 Ba	20.0 Aa
14	Ethanol	18.9 Aa	26.7 Aa	23.3 Aa
	Ethyl ether	14.4 Aa	24.4 Aa	21.1 Aa
	Hot water	27.8 Aa	25.6 Aa	17.8 Aa
	Water	23.3 Aa	24.4 Aa	18.9 Aa
	Citric acid	28.9 Aa	31.1 Aa	32.2 Aa
	Mechanical	18.9 Aa	21.1 Aa	16.7 Aa
	Control	16.7 Aa	32.2 Aa	24.4 Aa
21	Ethanol	30.0 Aa	27.8 Aa	23.3 Aa
	Ethyl ether	20.0 Aa	26.7 Aa	21.1 Aa
	Hot water	27.8 Aa	27.8 Aa	17.8 Aa
	Water	24.4 Aa	24.4 Aa	18.9 Aa
	Citric acid	28.9 Aa	31.1 Aa	32.2 Aa
	Mechanical	21.1 Aa	21.1 Aa	16.7 Aa
	Control	24.4 Aa	32.2 Aa	24.4 Aa

[x] Upper case letters denote differences in rows and lower case letters indicate differences within columns and days post-stratification. Means followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's Mean Separation (n=3).
[y] Germination percentages determined at 7, 14, and 21 days.
[z] Stratification at 2 °C (35.5 °F) in moist peat moss for 0, 45, and 90 d.

CHAPTER IV

EVALUATION OF DROUGHT TOLERANCE OF SELECTED PROVENANCES OF *TAXODIUM*

Water is one of the most important factors affecting plant growth and productivity (Taiz and Zeiger, 1998). Any decrease in the availability of water has implications for processes from solute transport to photosynthesis (Taiz and Zeiger, 1998). Pugnaire et al. (1999) defined water stress as "the induction of turgor pressure below the maximal potential pressure…the magnitude of such stress is determined by the extent and duration of the deprivation." Plants regularly must withstand water deficits and have evolved physiological responses and ecological strategies that allow them to survive (Pugnaire et al., 1999). They can cope with water stress in two ways: 1) drought avoidance and 2) drought tolerance (Levitt, 1980).

Drought avoidance strategies involve adaptations that maximize the acquisition of available water or the restriction of plant processes to periods of higher water availability (Levitt, 1980). These strategies may include a greater allocation of biomass to roots, water storage, changes in leaf morphology, increased stomatal sensitivity, the use of alternative metabolic pathways (i.e. CAM and C_4 photosynthesis) and drought-induced dormancy (Pugnaire et al., 1999).

Drought tolerance strategies include osmotic adjustment and changes to cells' elastic properties (Pugnaire et al., 1999). Osmotic adjustment, or osmoregulation, seems to be the more common means of drought tolerance. Osmotic adjustment is defined as the active accumulation of solutes inside the cell, with the consequent lowering of water

potential (Pugnaire et al., 1999). Plants may accumulate solutes, allowing turgor and turgor-related processes to continue in periods of water stress (Pugnaire et al., 1999). There are many solutes that can act as osmolytes. However, the most common osmoregulators in plants are sugars and amino acids, proline in particular (Pugnaire et al., 1999). It is hypothesized that this is because of the convenience of storing these osmolytes in large molecules, such as starch and protein (which are osmotically inactive), and can serve other purposes in the cell and can be readily converted in times of stress (Pugnaire et al., 1999).

Urban surfaces and compacted soils frequently decrease the amount of water that infiltrates into the root zone of trees; moreover, trees must compete with turf and other vegetation for the available water (Zwack and Graves, 1998). Therefore, water deficit situations can be common in urban areas. Zwack and Graves (1998) also point out a need for "tree taxa that maintain landscape function during episodes of variable and adverse soil moisture". St. Hillaire and Graves (2001) suggested that a strategy for selecting ornamentals with "superior resistance to drought stress" was to select from populations native to relatively xeric habitats.

Taxodium distichum (L.) Rich. is a widely adaptable tree species for landscape use, tolerating both wet and dry soils, and air pollution (Cox and Leslie, 1988; Wasowski and Wasowski, 1997). Watson (1983) reports tolerance to varying nutrient availability conditions, a wide range of soil aeration levels, and somewhat extreme pH levels. It is fast growing, has reliable feathery foliage, and a nice form (Arnold, 2002; Cox and Leslie, 1988). Two varieties, var. *distichum* (baldcypress) and var. *imbricarium* (Nutt.)

Croom (pondcypress), have fairly good fall color some years, while var. *mexicana* Gordon (Montezuma cypress) remains semi-evergreen (Arnold, 2002). It is an extremely long-lived tree, with a life span of up to 700 years possible (Cox and Leslie, 1988). All of these factors allow *T. distichum* to tolerate many environmental stresses, making it a promising choice for urban landscapes. However, there are a few limitations to this species. While it is tolerant of substantial soil salts, it tends to defoliate when leaves come into contact with salty irrigation water, tends to develop chlorosis on sites with high pH, and has a tendency to "brown out" in periods of extended or severe drought (Arnold, 2002).

The purpose of this study was to determine if there is a geographic basis for drought tolerance in *Taxodium* and to evaluate selected provenances in an effort to select those which yield individuals that are most adaptable/tolerant to this environmental stress.

Materials and Methods

Open-pollinated family identity was coded with four alphanumeric characters. The first two letters signify the general geographic origin of the mother tree. 'MX' signifies south Texas and Mexico, 'TX' signifies central Texas, and 'EP' denotes the southeastern U.S. The numeral is unique to an open-pollinated family from a given geographic area. The final letter indicates the taxonomic variety. 'M' indicates that the open-pollinated family belongs to the variety *mexicanum*, 'D' indicates var. *distichum*, and 'I' indicates var. *imbricarium*.

Screening 1

Thirteen open-pollinated families of *Taxodium distichum* were collected in the late summer and fall of 2003. Seeds were collected off a single mother tree at several locations (Table 5, Fig. 1) representing the ecophysiographic variation throughout the species' range. After collection, seeds were stratified for 90 d at 2 ºC. Localities representing 'normal' seed sources (mesic eastern U.S. sites), as well as sites representing more xeric environmental conditions (western U.S. and Mexican sites) were sampled. Seeds were planted in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with medium vermiculite (Sun Gro Horticulture, Bellevue, Wash.) on 12 Mar. 2004, and germinated before the end of Apr. 2004. On 8-10 Apr. 2004, 200 seedlings of each open-pollinated family were transplanted into 9.6 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with 3 pine bark : 1 coarse perlite (by volume) substrate amended with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled-release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ micronutrient fertilizer 0N-0P-0K-6Ca-3Mg-12S-17Fe (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), and 4.15 $kg \cdot m^{-3}$ $CaMgCO_3$ (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown outdoors under 55% light exclusion in a nursery area and irrigated by hand as needed.

Initial drought tolerance evaluations were conducted in a greenhouse beginning 6 June 2005. Containers were arranged in a completely randomized design. Plants were subjected to a regime of decreasing irrigation frequency, beginning with a daily watering, followed by a 2 d interval between irrigations, then a 3 d period, etc. The

study was terminated 8 Aug. 2005, after the 10 d interval between irrigations. Plant height and trunk diameter, as well as shoot and root dry mass were taken on the last day of the experiment to evaluate plant growth and biomass partitioning. Height and diameter measurements were taken at the initiation of the experiment and at its end. Pre-dawn xylem water potential was measured just before irrigation at the end of each dry down cycle using a pressure chamber (Model 610, PMS Instrument Company, Albany, Ore.). Volumetric water content of five containers chosen at random was measured hourly using dielectric soil moisture probes (Decagon Devices, Inc., Pullman, Wash.).

Growth and morphology (height, trunk diameter, dry mass, root:shoot ratio and time to mortality), as well as xylem water potential data were analyzed using univariate analysis in the GLM procedure of SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Hierarchical cluster analysis using squared euclidean distance as a measure and the nearest-neighbor method in SPSS utilizing pre-dawn water potentials and mortality was used to generate dendrograms.

Table 5. Localities of mother trees providing seeds of open-pollinated families of *Taxodium distichum* collected in the late summer and fall of 2003.

Family	Latitude	Longitude	Locale
MX1M	25º52′48″N	97º27′0″W	Southmost, TX
MX3M	19º30′0″N	98º54′36″W	Bolleros, MX
MX4M	27º51′0″N	101º7′48″W	Rio Sabinas, MX
MX5M	26º4′12″N	97º54′36″W	Progreso, TX
TX1D	29º57′36″N	98º48′0″W	Guadalupe River, Waring, TX
TX2D	30º4′12″N	99º17′24″W	Guadalupe River, Hunt, TX
TX5D	29º9′36″N	99º28′12″ºW	Sabinal River, TX
TX6D	29º0′36″N	98º34′48″W	Atascosa River, TX
TX8D	29º52′48″N	97º55′48″W	San Marcos River, TX
EP1D	32º20′24″N	94º42′0″W	Lake Cherokee, TX
EP2D	29º48′0″N	91º47′24″W	Iberia Parish, LA
EP4D	30º36′0″N	87º54′36″W	Mobile Bay,AL
EP5I	30º27′0″N	88º6′36″W	Fowl River, AL
EP6D	30º24′36″N	88º54′0″W	Biloxi, MS
EP8D	31º33′36′ N	91º26′24″W	Mississippi River, LA

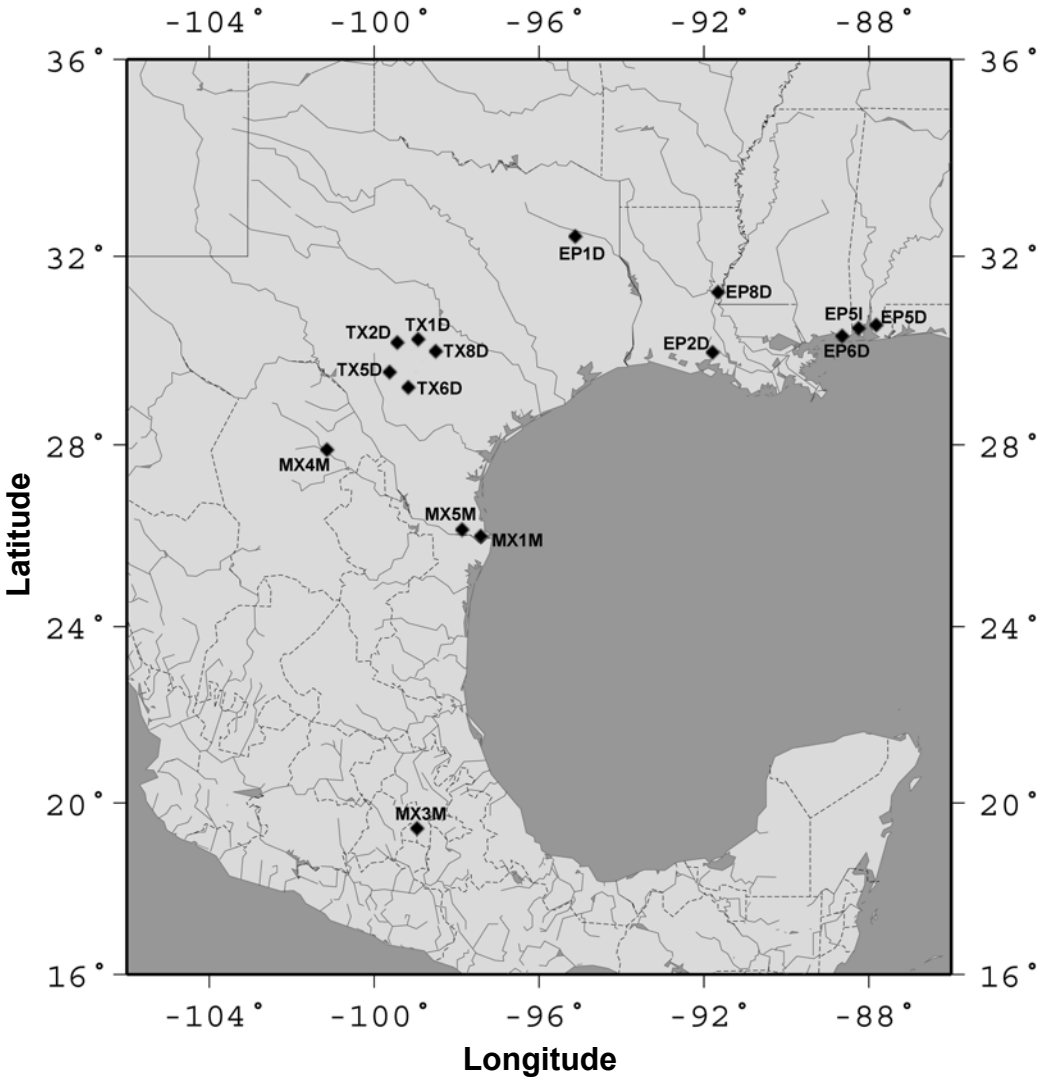

Figure 1. Locations of mother trees providing seeds for open-pollinated families of *Taxodium distichum* used in drought tolerance screenings. Symbols represent collection sites and open-pollinated family identity is indicated beside symbol.

Screening 2

Four open-pollinated families of *Taxodium distichum* were selected for screening in the spring of 2006 (Table 5, Fig. 1). Families were selected to represent the ecophysiographic variation between the "Mexican" type populations (extreme south Texas and Mexico, Family MX5M) and those from central Texas (Families TX1D, TX2D, TX5D) because of the superior performance of genotypes from these regions in the initial screening. Cuttings off multiple trees per family from a stock block maintained in the field in College Station, Texas were rooted on 20 March 2006. Cuttings were treated with a 8000 $mg \cdot L^{-1}$ IBA and 4000 $mg \cdot L^{-1}$ NAA dip (Dip 'n Grow, Inc., Clackamas, Ore.) and were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture, Bellevue, Wash.). Rooted cuttings were planted on 12 May 2006 into 2.5 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with calcined clay (Oil-Dri Corp. of America, Alpharetta, Ga.) amended with 6.53 $kg \cdot m^{-3}$ of 15N-3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ micronutrient fertilizer 0N-0P-0K-6Ca-3Mg-12S-17Fe (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), 4.15 $kg \cdot m^{-3}$ $CaMgCO_3$ (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown in a greenhouse with 26.7 ºC / 23.9 ºC day/night temperature set points. Typical light levels as measured in mid-afternoon on 30 Aug. 2006 was 702 $\mu mol \cdot m^{-2} \cdot s^{-2}$ PAR.

Drought tolerance evaluations were conducted in the greenhouse beginning on 4 September 2006. Containers were arranged in a completely randomized design. Plants

were subjected to an acute drought stress by withholding water. Plants within a family were harvested when at least half of the treated plants in that family showed foliar death. Plant height and trunk diameter, as well as shoot and root fresh and dry mass were measured at the end of the experiment to evaluate plant growth and biomass partitioning. Pre-dawn xylem water potential was measured at harvest using a pressure chamber. Volumetric water content at harvest of all the containers was calculated from fresh and dry masses and volume measurements of the substrate. The mass of the water present in the containers was calculated as the difference between the fresh and dry mass of the substrate. The density of water was assumed to be 1.0, allowing the easy conversion from mass to volume. The volumetric water content of the substrate was then calculated with the following formula: $V\% = (volume_{water} / volume_{subsrate}) * 100$. Growth and morphology (height, trunk diameters, dry masses, root:shoot ratios, shoot and substrate water contents, and days to mortality), as well as xylem water potential data, were analyzed using univariate analysis in the GLM procedure of SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.).

Pressure-Volume Curves

In spring 2006, three open-pollinated families of *Taxodium distichum* were selected which represent the ecophysiographic variation throughout the tested species' range (Table 5, Fig. 1). The genotypes used represented seed sources from the southeastern U.S. (family EP8D, Vidalia, La.), central Texas (family TX6D, Atascosa River, Texas), and the Rio Grande Valley of Texas (family MX5M, Progresso, Texas). Cuttings off multiple trees per family from a stock block maintained in the field in

College Station, Texas were rooted on 20 Mar. 2006. Cuttings were treated with a 8000 $mg \cdot L^{-1}$ IBA and 4000 $mg \cdot L^{-1}$ NAA dip (Dip 'n Grow, Inc., Clackamas, Ore.) and were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture, Bellevue, Wash.). Rooted cuttings were transplanted on 12 May 2006 into 2.5 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with calcined clay (Oil-Dri Corp. of America, Alpharetta, Ga.) amended with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ micronutrient fertilizer 0N-0P-0K-6Ca-3Mg-12S-17Fe (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), and 4.15 $kg \cdot m^{-3}$ $CaMgCO_3$ (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown in a greenhouse with 26.7 ºC / 23.9 ºC day/night temperature set points. Typical light levels as measured in mid-afternoon measured on 30 Aug. 2006 were 702 $\mu mol \cdot m^{-2} \cdot s^{-2}$ PAR. Containers were arranged in a completely randomized design on a single bench during growth and were irrigated as needed.

On 18 Sept. 2006, three rooted cuttings from each of the three selected families were used to perform a pressure-volume analysis as described by Turner (1988). Care was taken to ensure that shoots had comparable amounts of foliage per shoot, as is suggested by Neufeld and Teskey (1986). Shoots were cut and allowed to rehydrate to full turgor in distilled water for 18 h in the dark at 5.5 ºC. Fresh mass (FW) of each cutting was measured followed immediately by its xylem water potential beginning at the end of the rehydration period and then every 30 min thereafter until xylem water potential reached -4.0 MPa. The initial fresh mass is referred to from here on as TW.

Dry mass (DW) of each cutting was also measured. Relative water content (RWC) of the cuttings was calculated using the formula RWC = [(FW-DW)/(TW-DW)] *100.

Water contents, fresh mass to dry mass ratios, and xylem water potential data were analyzed and parameter estimates generated using univariate analysis in the GLM procedure of in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.).

Results and Discussion

Screening 1

There were significant differences in pre-dawn water potentials among families after 5, 6, 7, 8, and 10 d of imposed drought ($P \leq 0.05$) (Fig. 2). Additionally, there was a significant difference in the mean survivable water deficit among families ($P \leq 0.05$) (Fig. 3). A hierarchical cluster analysis of the families based on pre-dawn xylem water potentials from the 5, 6, and 7 d drought periods (Fig. 2) and the survivable drought period of each family (Fig. 3) generated a dendrogram showing the relationship among families based on their performance in this screening (Fig. 4). The parameters utilized in this analysis were selected because the open-pollinated families showed the most separation during the 5, 6, and 7 d drought periods. After the 7 d drought period, some of the individuals exhibited canopy death, leading to a less negative pre-dawn xylem water potential measurement. Time to canopy death was selected as a parameter in an effort to account for this phenomenon. The dendrogram divides the families into two main groups. The eastern populations all fall into one group (Families EP2D, EP4D, EP5I, EP6D, and EP8D) and the Texas (Familes MX5M, TX2D, and TX8D) and Mexican (Families MX3M and MX4M) populations fall into the other. The exception is

the open-pollinated family (TX6D) from Poteet, TX, which clusters with the eastern populations. Within the cluster of eastern populations there are two groups. The first includes populations that all belong to the variety *distichum*, and the second includes families from both variety *distichum* (TX6D, Poteet, TX) and var. *imbricarium* (EP5I, Fowl River, AL and EP6D, Biloxi, MS). No significant differences in root to shoot ratios were found (P=0.372).

The clustering of the families suggests that there is a geographic component to variation in drought tolerance of *Taxodium distichum*. The observed geographic pattern is what might be expected. The open-pollinated families from eastern localities were less tolerant of drought than open-pollinated families from western populations. This is likely due to a general trend in decreasing rainfall as we move from east to west in the southern U.S. The implication is that when selecting genotypes for more xeric situations, an effort should be made to obtain genotypes from central Texas or Mexico. Additionally, open-pollinated families from south Texas and Mexico appeared less stressed at times of xylem water potential measurement, although no data was taken on general appearance because of its subjective nature.

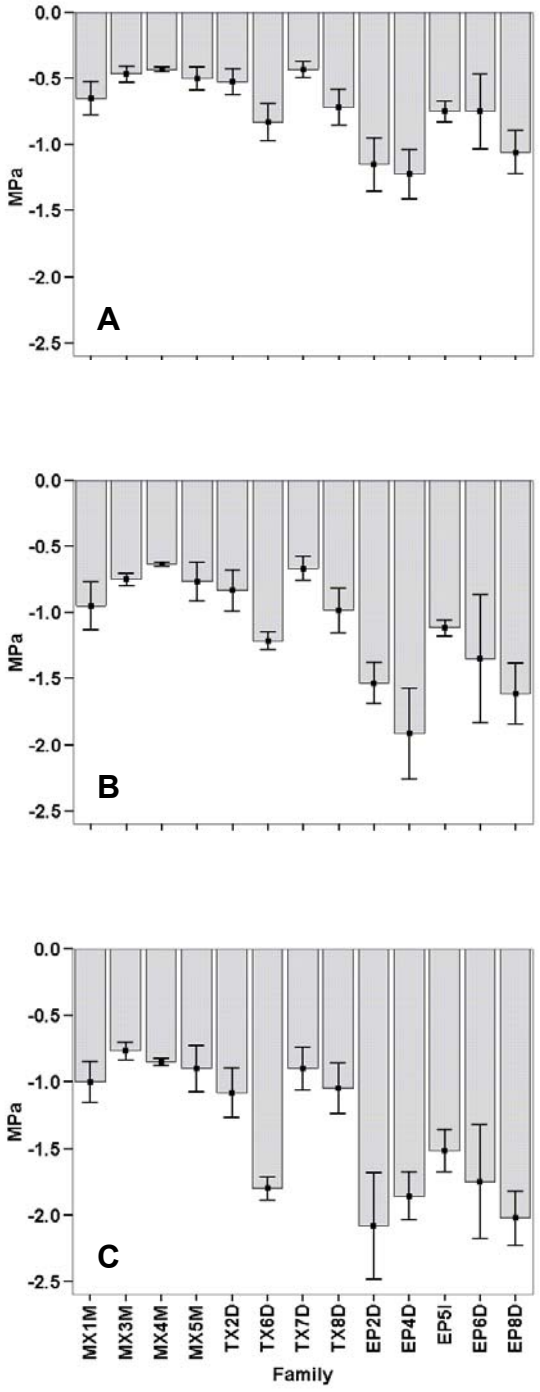

Figure 2. Pre-dawn xylem water potentials of 13 open-pollinated families of *Taxodium distichum* after 5 (A), 6 (B), and 7 (C) d drought periods. Values represent means of three observations ± standard errors.

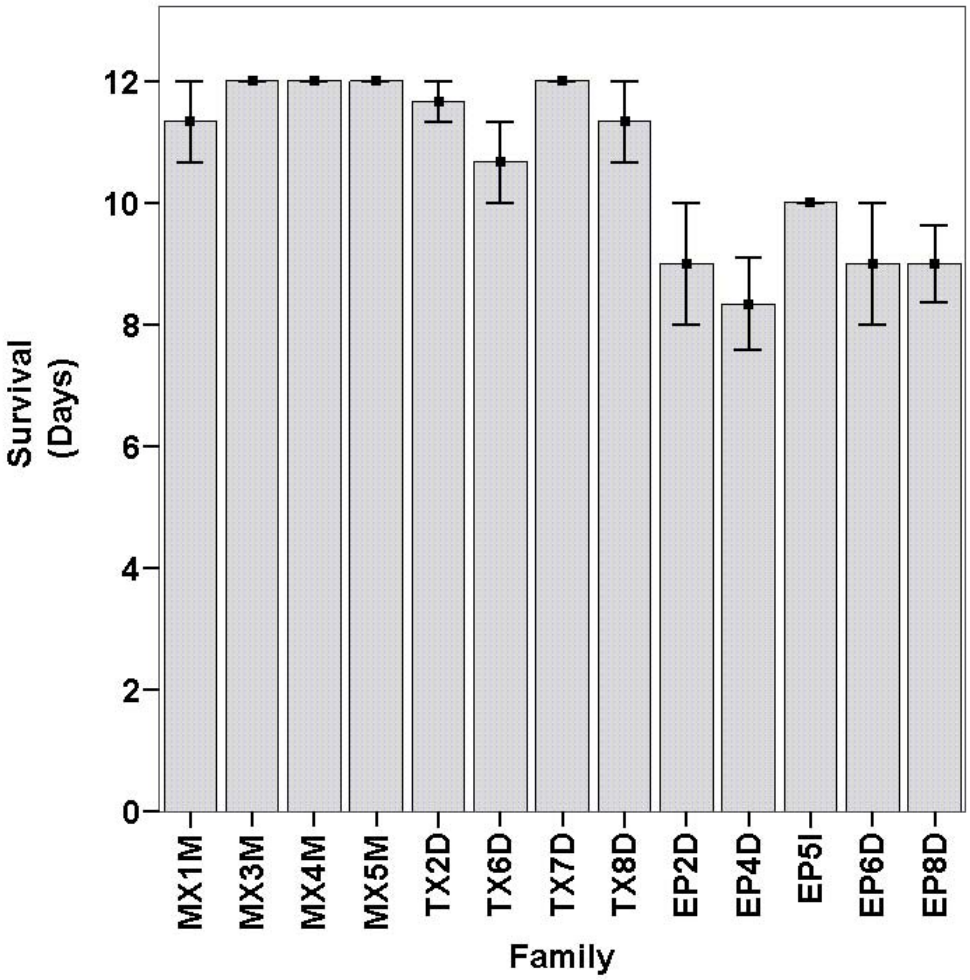

Figure 3. Maximum survivable drought period of thirteen open-pollinated families of *Taxodium distichum*. Values represent means of three observations ± standard errors.

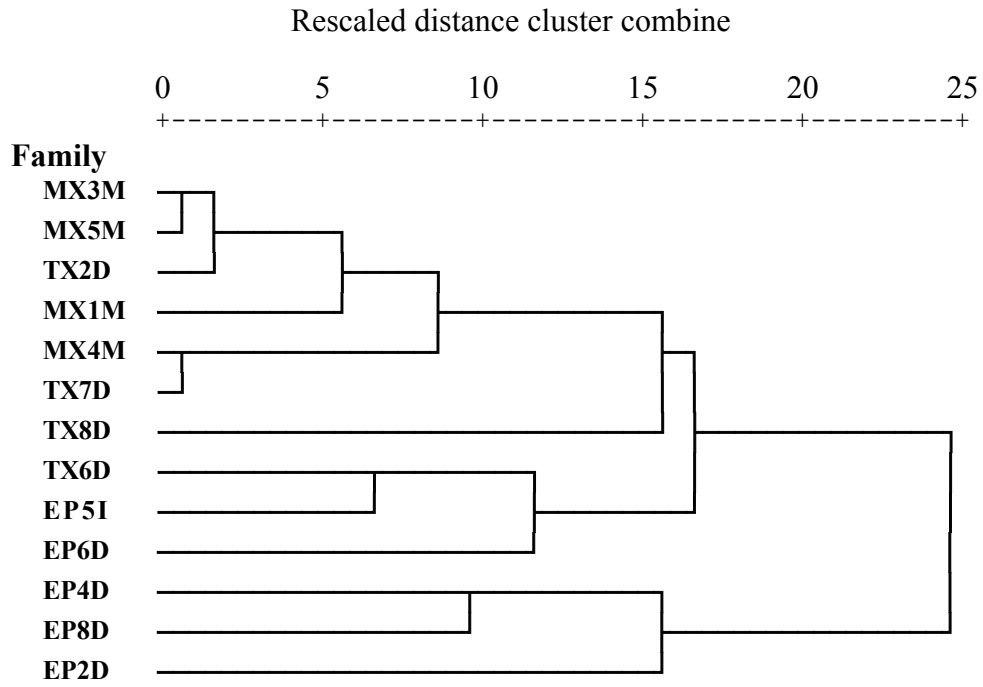

Figure 4. Dendrogram generated by a hierarchical cluster analysis based on pre-dawn xylem water potentials from the 5, 6, and 7 d drought periods and the maximum survivable drought period showing the relationship among thirteen open-pollinated families of *Taxodium distichum*.

Screening 2

There were significant ($P \leq 0.05$) treatment effects related to water deficits among the families in all the parameters measured. Well-irrigated control plants did not differ significantly among open-pollinated families. Treatment plants from family MX5M had 50% survival after 11 d without irrigation, while families TX1D, TX2D, and TX7D had 50% survival after only 8 d. Volumetric water content of the substrate at the time of harvest was significantly lower ($P \leq 0.01$) for family MX5M than the other families, which did not differ significantly from each other (Fig. 5). Shoot water content at harvestwas significantly lower ($P \leq 0.01$) for family MX5M than the other families (Fig. 6). Shoot water content of family TX1D was significantly higher ($P \leq 0.05$) than all other families (Fig. 6). Families TX2D and TX5D did not differ from each other, but did differ from families MX5M and TX1D. An estimated substrate water depletion rate was calculated by dividing the difference between the mean substrate volumetric water content for the well watered control plants and the observed substrate volumetric water content for the treatment plants by the number of days to harvest. Family MX5M showed a lower estimated water depletion rate than the other families ($P \leq 0.01$) (Fig. 7). No significant difference was found between the root to shoot ratios of the families ($P = 0.11$) (data not shown).

These results support the observation in the initial screenings that the Mexican families appeared less water stressed compared to the central Texas families. After similar drought periods, the Mexican genotypes had a higher water content per unit dry mass. They were able to withstand longer droughts than central Texas families because

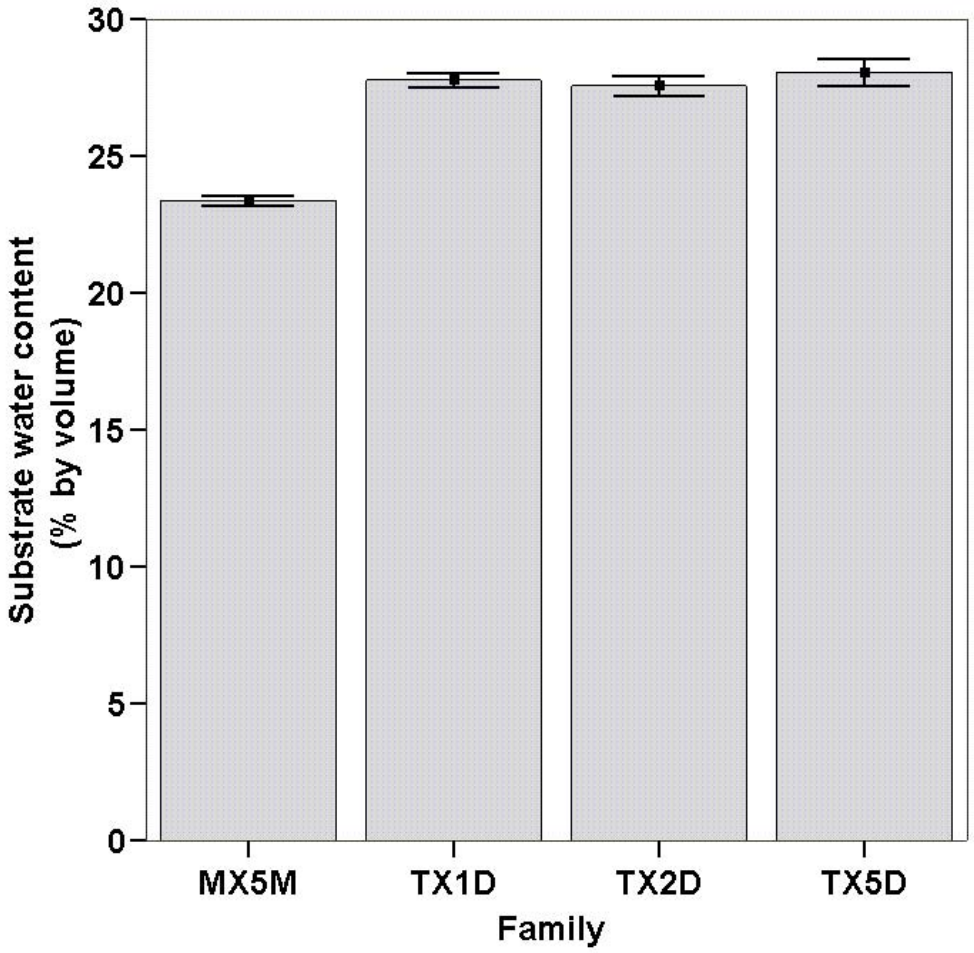

Figure 5. Volumetric water content of the substrate at the time of harvest for four open-pollinated families of *Taxodium distichum*. Values represent means for eight observations ± standard errors.

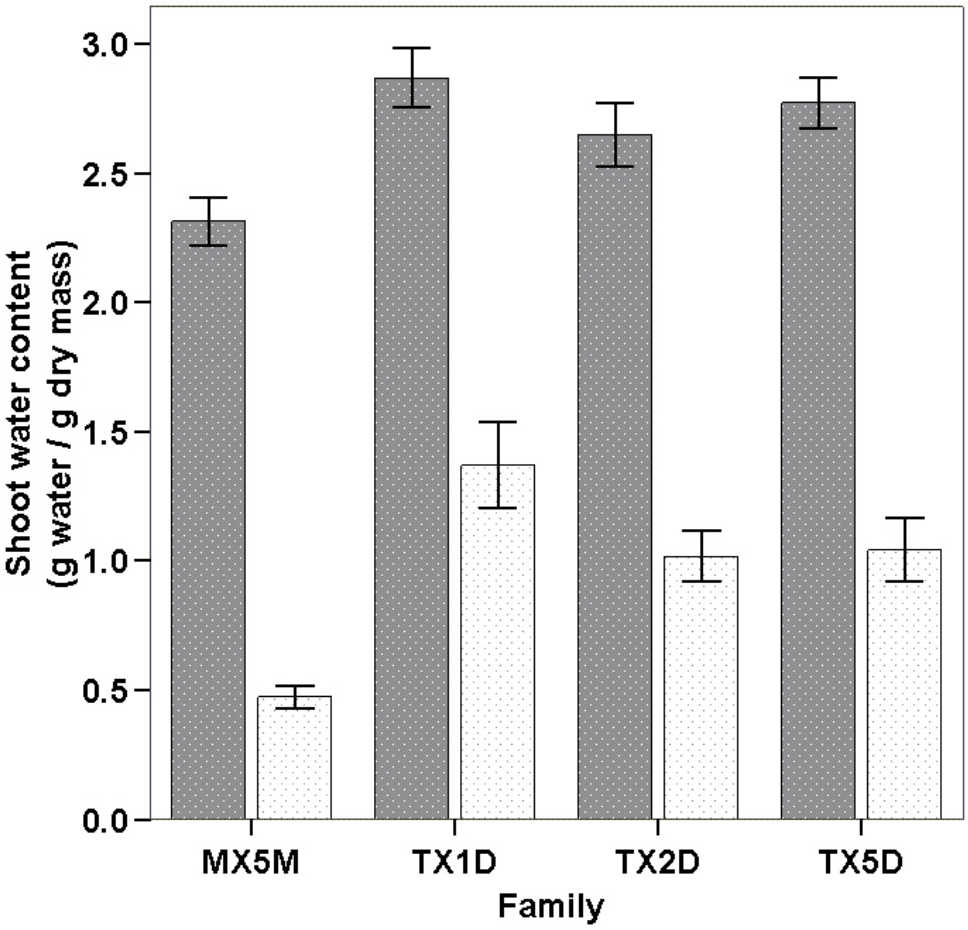

Figure 6. Water content of shoots based on dry weight at the time of harvest for four open-pollinated families of *Taxodium distichum*. Values represent means for eight observations ± standard errors. Dark bars represent control plants, while light bars represent drought treatment plants.

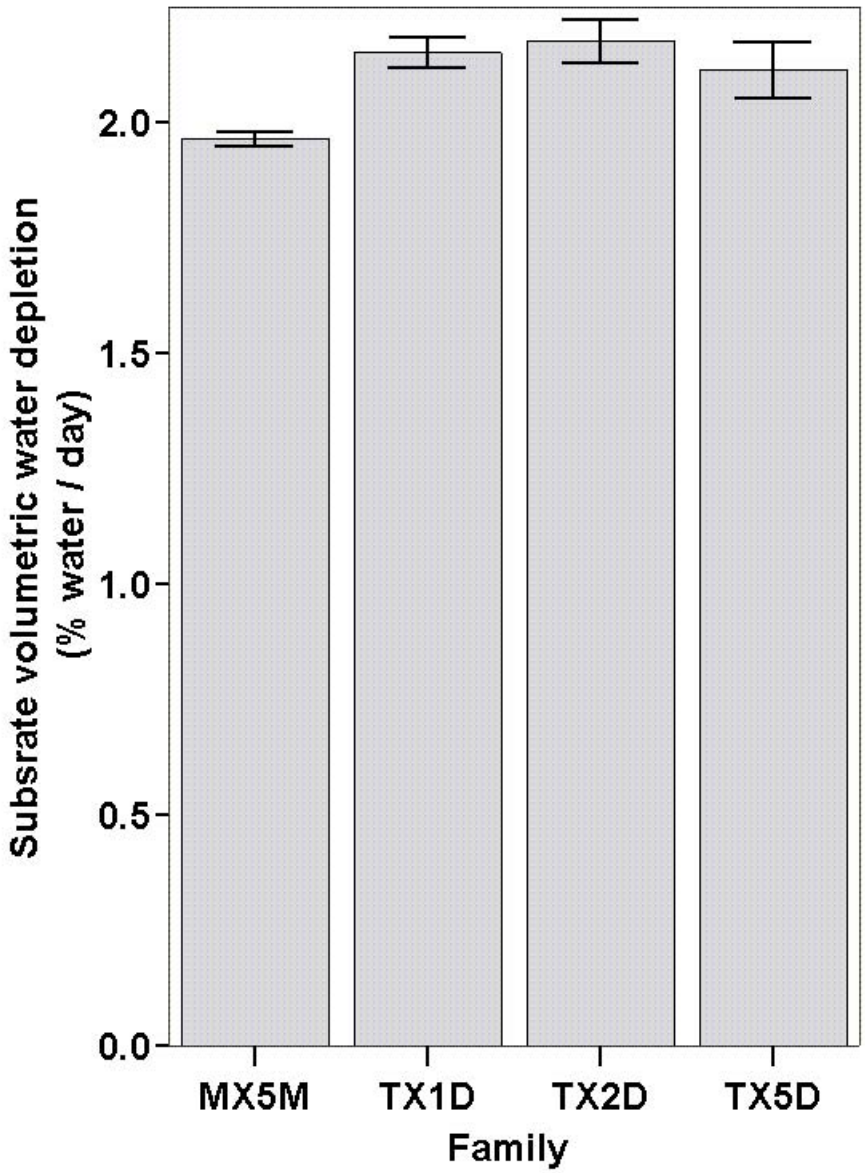

Figure 7. Substrate volumetric water content loss rate for four open-pollinated families of *Taxodium distichum*. Values represent means for eight observations ± standard errors.

they were able to survive at lower substrate volumetric water contents. They also removed water from the substrate at a lower rate (Fig. 7), implying that they are better at controlling water loss from their shoots. The Mexican genotype was also able to extract more water from the substrate (Fig. 5). This suggests that *Taxodium* may utilize both drought tolerance and drought avoidance as mechanisms for resisting drought stress.

Pressure-Volume Analysis

Pressure-volume analysis allows many plant-water parameters to be derived including: total water content, turgid/dry mass ratio, relative water content, apoplastic and symplastic water contents, relative symplastic water contents, osmotic pressure at full and zero turgor, relative water content at zero turgor, bulk moduli of elasticity, and tissue moisture release curves (Turner, 1988). It also provides the needed parameters to create a Höfler diagram (Turner, 1988). Pressure-volume curves have been used extensively to examine many aspects of plant-water relations by numerous authors (Fan et al., 1994; Roberts et al., 1981; White et al., 2001). Li (1998) utilized pressure-volume analysis to compare leaf water relations of *Eucalyptus microtheca* F. Muell. provenances.

Relative water content of each of the samples was calculated for each measurement point. The rate of relative water content (RWC) loss was significantly different among the eastern family (EP8D) and both the central Texas (TX6D) and south Texas (MX5M) families ($P \leq 0.001$) (Fig. 8, Table 6). The families from Texas did not significantly ($P \geq 0.05$) differ in RWC loss rates. The difference in rate of RWC loss among the three families in the study supports the findings of the previous screenings of

the open-pollinated families. Eastern families tended to desiccate (decrease in RWC) more rapidly than south Texas families, while families from central Texas tended to be intermediate.

The relationship between the plant water potential and RWC is referred to as the water potential isotherm or the moisture release curve for a tissue (Turner, 1988). It has been used to determine the drought resistance characteristics of various species (Jones et al., 1981). Xylem water potential also differed significantly in response to decreasing RWC among all three families (Fig. 9, Table 6). The south Texas family (MX5M) showed the largest decrease in xylem water potential per unit change in RWC, followed by the central Texas family (TX6D) and then the eastern family (EP8D). The south Texas family has the steepest moisture release curve (Fig. 9), implying that its tissues retain water more tightly than the other families. The eastern family has the shallowest moisture release curve implying that the water in these plants is held the least tightly. The moisture release curve of the central Texas family was intermediate.

Xylem water potential of family MX5M decreases slightly faster than those of either of the other two families (Fig. 10, Table 7). The fresh mass to dry mass ratio decreases more rapidly in the eastern family (EP8D) compared to the Texas families (Fig. 11, Table 7). The turgid to dry mass ratio has been shown to correlate well with osmotic adjustment in some species (Turner, 1988). This is logical because the higher the ratio is the more water per unit dry mass the plant contains. However, this may be due to higher osmotic potentials or to more elastic cells.

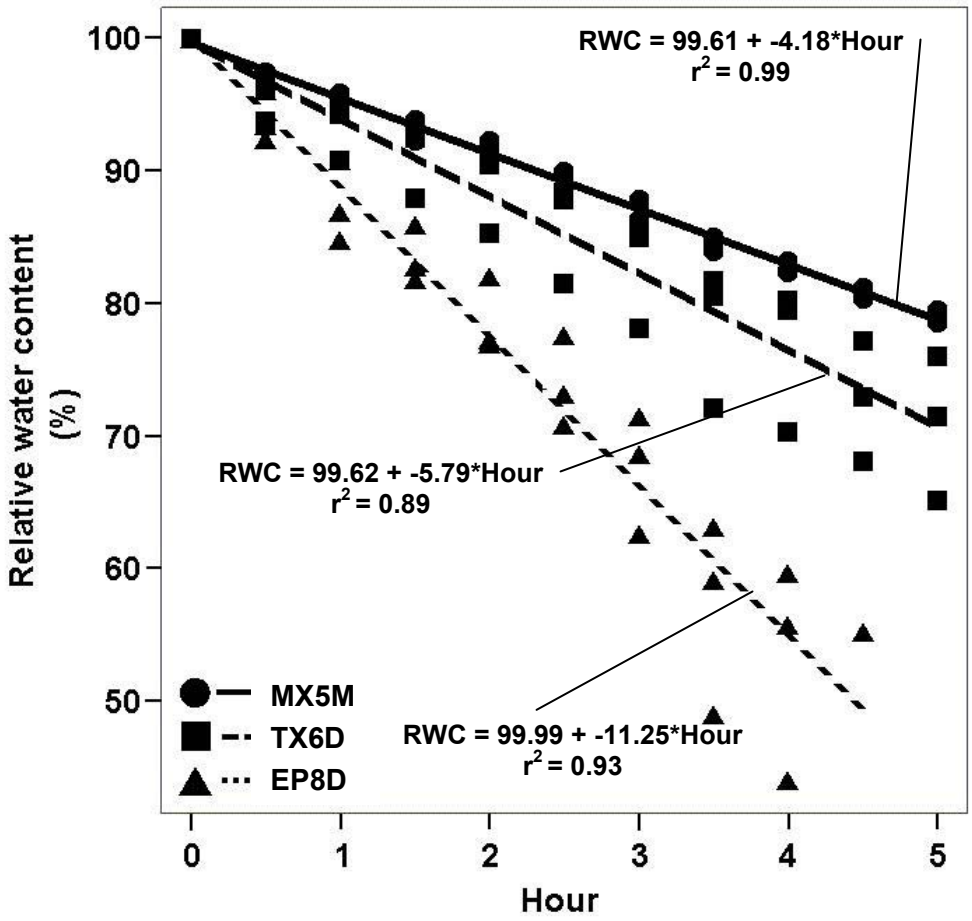

Figure 8. Rate of relative water content loss for three open-pollinated families of *Taxodium distichum*; Family EP8D (Louisiana), Family TX6D (central Texas), and Family MX5M (south Texas). Symbols represent individual observations, while lines are best fit linear regressions.

Table 6. Analysis of covariance table for the rates of shoot relative water content decrease, xylem water potential decrease, and change in the fresh to dry mass ratio in three open-pollinated families of *Taxodium distichum*.

Figure	Measured Characteristic	r^2	Source	Significance
Fig. 8	Relative water content	0.94	Model	<0.001
			Intercept	<0.001
			Family	0.958
			Time	<0.001
			Family X Time	<0.001
Fig. 9	Xylem water potential	0.83	Model	<0.001
			Intercept	<0.001
			Family	<0.001
			RWC	<0.001
			Family X RWC	<0.001
Fig. 10	Fresh : Dry Mass Ratio	0.72	Model	<0.001
			Intercept	<0.001
			Family	<0.001
			Time	<0.001
			Family X Time	<0.001
Fig. 11	Xylem water potential	0.84	Model	<0.001
			Intercept	<0.001
			Family	0.063
			Time	<0.001
			Family X Time	0.039

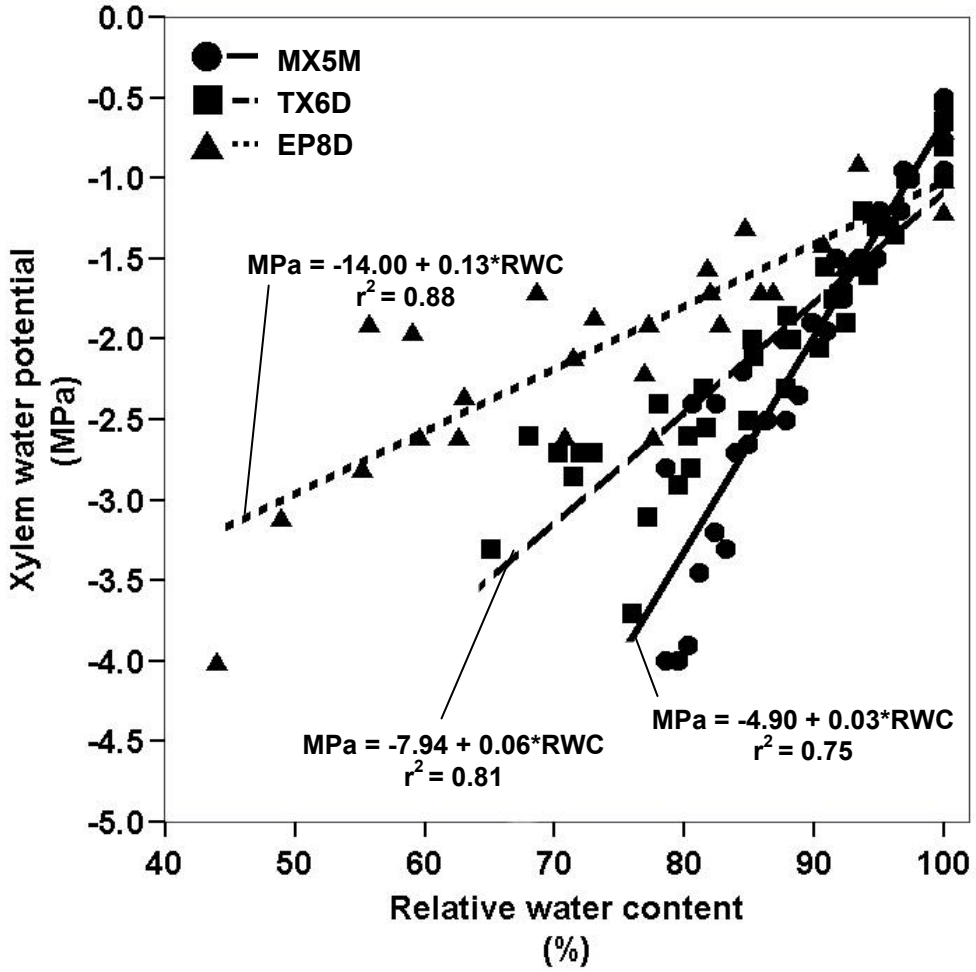

Figure 9. Moisture release curve for three open-pollinated families of *Taxodium distichum.* Family EP8D (Louisiana), Family TX6D (central Texas), and Family MX5M (south Texas). Symbols represent individual observations, while lines are best fit linear regressions.

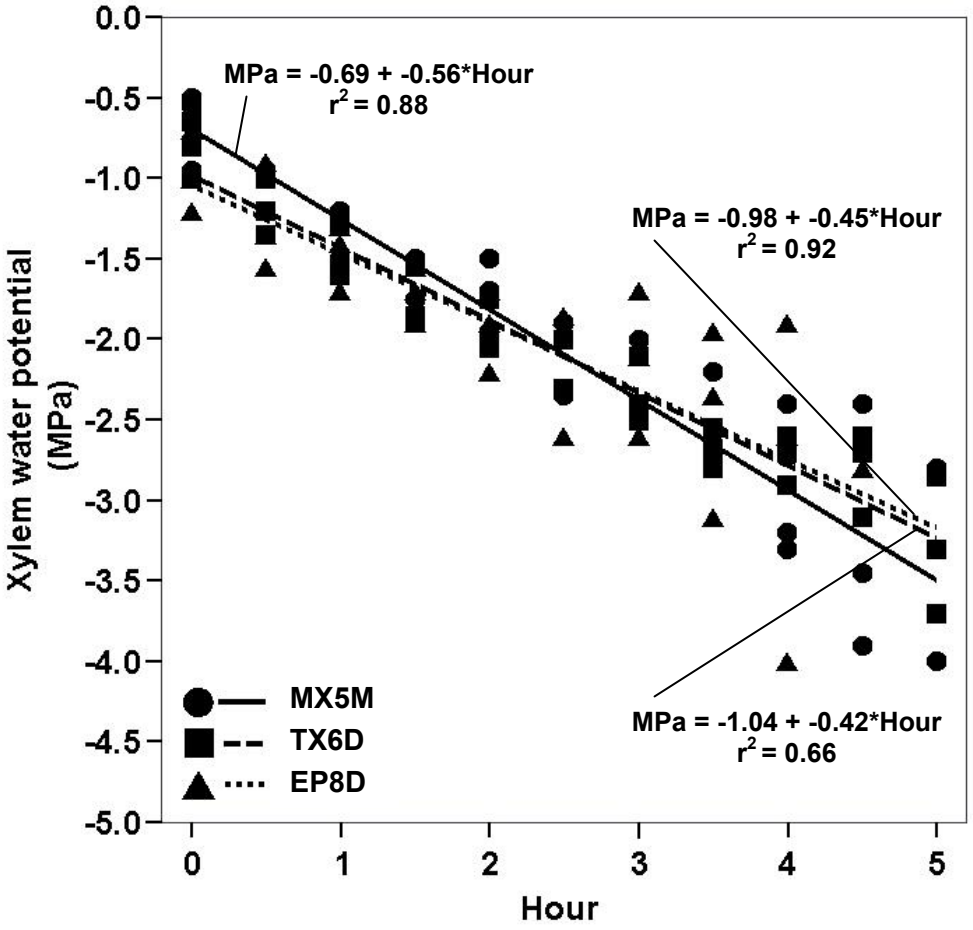

Figure 10. Rate of xylem water potential decrease for three open-pollinated families of *Taxodium distichum*. Family EP8D (Louisiana), Family TX6D (central Texas), and Family MX5M (south Texas). Symbols represent individual observations, while lines are best fit linear regressions.

Table 7. Parameter estimates for the rates of shoot relative water content decrease, xylem water potential decrease, and change in the fresh to dry mass ratio in three open-pollinated families of *Taxodium distichum*. The values in column b are the parameter estimates generated by analysis of covariance.

				95% Confidence Interval	
Figure	**Measured Characteristic**	**Parameter**	**b**	**Lower Bound**	**Upper Bound**
Fig. 8	Relative water content	Family(MX5M)	99.61	97.63	101.59
		Family(TX6D)	99.62	97.64	101.61
		Family(EP8D)	99.99	97.87	102.11
		Family(MX5M) X Time	-4.18	-4.85	-3.51
		Family(TX6D) X Time	-5.79	-6.47	-5.12
		Family(EP8D) X Time	-11.25	-12.10	-10.39
Fig. 9	Xylem water potential	Family(MX5M)	-14.00	-15.62	-12.38
		Family(TX6D)	-7.94	-9.00	-6.88
		Family(EP8D)	-4.90	-5.55	-4.26
		Family(MX5M) X RWC	0.13	0.11	0.15
		Family(TX6D) X RWC	0.06	0.05	0.08
		Family(EP8D) X RWC	0.03	0.03	0.04
Fig. 10	Fresh : Dry Mass Ratio	Family(MX5M)	3.33	3.18	3.47
		Family(TX6D)	3.36	3.22	3.51
		Family(EP8D)	4.11	3.96	4.26
		Family(MX5M) X Time	-0.09	-0.14	-0.04
		Family(TX6D) X Time	-0.14	-0.18	-0.09
		Family(EP8D) X Time	-0.33	-0.39	-0.28
Fig. 11	Xylem water potential	Family(MX5M)	-0.69	-0.91	-0.48
		Family(TX6D)	-0.98	-1.19	-0.76
		Family(EP8D)	-1.04	-1.27	-0.81
		Family(MX5M) X Time	-0.56	-0.63	-0.48
		Family(TX6D) X Time	-0.45	-0.52	-0.37
		Family(EP8D) X Time	-0.42	-0.51	-0.33

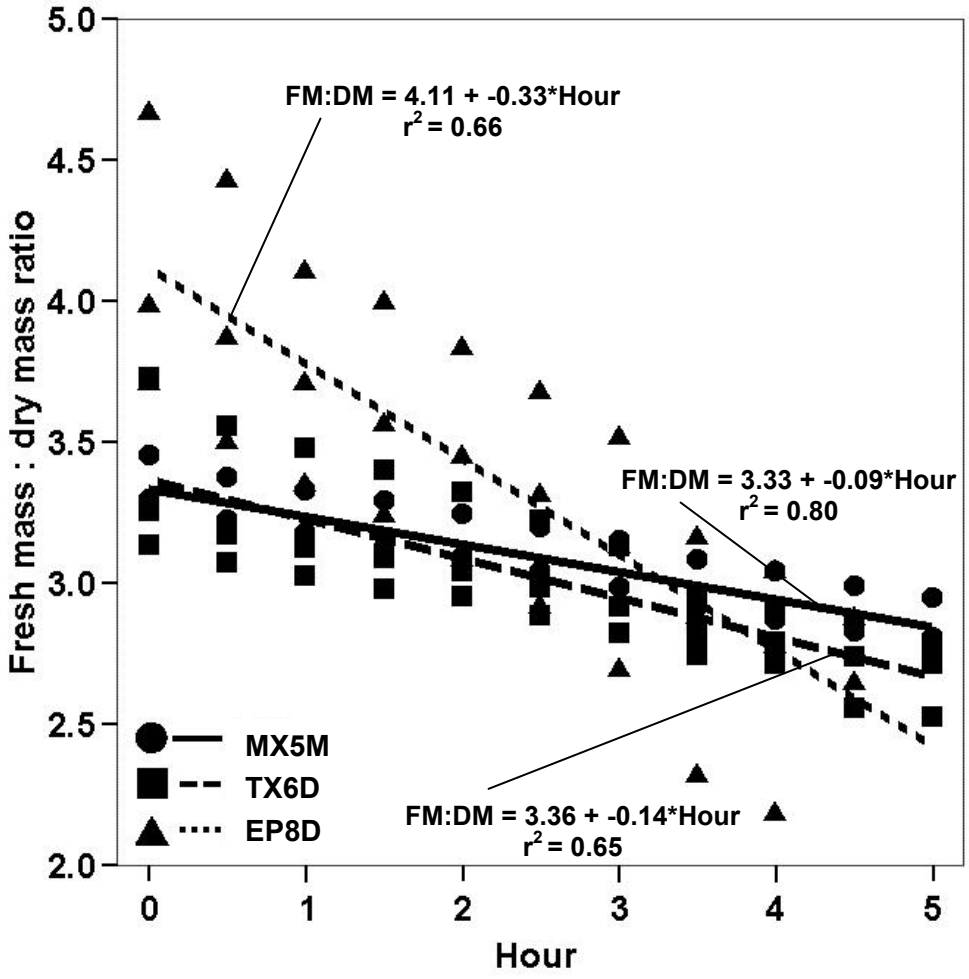

Figure 11. Rate of change in fresh to dry mass ratio for three open-pollinated families of *Taxodium distichum.* Family EP8D (Louisiana), Family TX6D (central Texas), and Family MX5M (south Texas). Symbols represent individual observations, while lines are best fit linear regressions.

Differences in the rate of change in the ratio between fresh mass and dry mass over time can indicate the concentration of osmolytes in the leaf tissue. This may be the case here. As the tissues began to desiccate, the south Texas (MX5M) family shows only a slight decrease in the fresh to dry mass ratio and the slope of the line does not differ significantly from 0 (Fig. 11, Table 7). The central Texas family (TX6D) also shows only a slight decrease in fresh to dry mass ratio during desiccation. Although the slope of the regression line for this family does not differ significantly from that of the south Texas family (MX5M), it is less than zero (Fig. 11, Table 7). The slope of the linear regression for the eastern family (EP8D) differs from those of both Texas families and is significantly less than zero (Fig. 11, Table 7). This means that as the tissue desiccates it holds relatively less water than it did while it was wetter. This would not be expected if the plant was utilizing osmotic adjustment as a strategy to resist drought, which would likely give the opposite result.

Taxodium distichum likely relies on both drought avoidance and drought tolerance strategies to deal with drought stress. It seems to avoid drought by limiting water loss from the shoots rather than increasing biomass partitioning to the roots. This is evident in the slower desiccation rates observed in the pressure-volume analysis and the lower substrate water removal rate in the south Texas and Mexican families, while maintaining a similar root to shoot ratios across all open-pollinated families. Drought tolerance seems to take the form of osmotic adjustment, as the moisture release curves and fresh to dry mass ratios indicate. Variation in these traits seems to have a geographic component. The occurrence of these important traits appears to increase as

populations are sampled from east to west in the U.S. and then south into Mexico, following a general environmental gradient of decreasing precipitaion. Similar patterns were observed by Li (1998) in *Eucalyptus* L'Her. provenances and St. Hilaire and Graves (2001) in *Acer saccharum* Marsh. seedlings. Shoemake et al. (2004) report that in *Platanus occidentalis* L., genotypes from xeric regions performed better than those from mesic areas when grown on a xeric site. The implication is that when choosing *Taxodium* for use in more xeric conditions care should be taken to select western genotypes.

CHAPTER V

EVALUATION OF ALKALINITY TOLERANCE OF SELECTED PROVENANCES OF *TAXODIUM*

Drought, salinity, and alkaline soils are common problems faced by many arborists, urban foresters, landscapers and homeowners. Kelsey and Hootman (1990) found that many urban street tree planter soils could be classified as saline or sodic, soil types that usually occur in arid or semi-arid areas with a higher evapotranspiration than precipitation rate. In many parts of the United States, drought and irrigation restrictions are becoming more common (Beeson et al., 2004).

Taxodium distichum (L.) Rich. is a widely adaptable tree species for landscape use, tolerating both wet and dry soils, and air pollution (Cox and Leslie, 1988; Wasowski and Wasowski, 1997). Watson (1983) reports tolerance to varying nutrient availability conditions, a wide range of soil aeration levels, and somewhat extreme pH levels. It is fast growing, has reliable feathery foliage, and a nice form (Arnold, 2002; Cox and Leslie, 1988). Two varieties, var. *distichum* (baldcypress) and var. *imbricarium* (Nutt.) Croom (pondcypress), have fairly good fall color some years, while var. *mexicana* Gordon (Montezuma cypress) remains semi-evergreen (Arnold, 2002). It is an extremely long-lived tree, with a life span of up to 700 years possible (Cox and Leslie, 1988). All of these factors allow *T. distichum* to tolerate many environmental stresses, making this a promising choice for urban landscapes. However, there are a few limitations to this species. While it is tolerant of substantial soil salt levels, it tends to defoliate when leaves come into contact with salty irrigation water, tends to develop

chlorosis on sites with high pH soils, and has a tendency to "brown out" in periods of extended or severe drought (Arnold, 2002).

The purpose of this study is to determine if there is a geographic basis for alkalinity tolerance in *Taxodium* and to evaluate provenances in an effort to select those that could yield individuals that are most adaptable/tolerant to these environmental stresses.

Materials and Methods

Open-pollinated family identity was coded with four alphanumeric characters. The first two letters signify the general geographic origin of the mother tree. 'MX' signifies south Texas and Mexico, 'TX' signifies central Texas, and 'EP' denotes the southeastern U.S. The numeral is unique to an open-pollinated family from a given geographic area. The final letter indicates the taxonomic variety. 'M' indicates that the open-pollinated family belongs to the variety *mexicanum*, 'D' indicates var. *distichum*, and 'I' indicates var. *imbricarium*.

Field Screening

Fourteen open-pollinated families of *Taxodium distichum* were collected in the late summer and fall of 2003. Seeds from a single mother tree at several locations (Table 8, Fig. 12) representing the ecophysiographic variation throughout the species range were collected and stratified (90 d at 2 ºC). Localities representing "normal" seed sources (mesic, acidic eastern U.S. sites), as well as sites representing more extreme environmental conditions (more xeric, alkaline western U.S. and Mexican sites) were sampled. Seeds were planted on 12 Mar. 2004, and germination took place before the

end of Apr. 2004. On 8-10 Apr. 2004, seedlings were transplanted into 2.5 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with 3 pine bark : 1 coarse perlite (by volume) mix amended with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled-release fertilizer (Osmocote® Plus, Scotts Company, Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ 0N-0P-0K-6Ca-3Mg-12S-17Fe micronutrient fertilizer (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), and 4.15 $kg \cdot m^{-3}$ $CaMgCO_3$ (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown under 55% light exclusion in a nursery area and irrigated as needed.

Table 8. Localities of 14 mother trees providing seeds of open-pollinated families of *Taxodium distichum* collected in the late summer and fall of 2003 used in alkalinity screenings.

Family	**Latitude**	**Longitude**	**Locale**	**pH[z]**
MX1M	25º52′48″N	97º27′0″W	Southmost, TX	7.8
MX2M	25º18′36″N	104º38′24″W	Rio Nazas, MX	na
MX4M	27º51′0″N	101º7′48″W	Rio Sabinas, MX	na
MX5M	26º4′12″N	97º54′36″W	Progreso, TX	7.8
TX2D	30º4′12″N	99º17′24″W	Guadalupe River, TX	7.2
TX6D	29º0′36″N	98º34′48″W	Atascosa River, TX	7.1
TX7D	29º46′12″N	98º8′24″W	Guadalupe River, TX	na
TX8D	29º52′48″N	97º55′48″W	San Marcos River, TX	7.5
EP1D	32º20′24″N	94º42′0″W	Lake Cherokee, TX	7.2
EP2D	29º48′0″N	91º47′24″W	Iberia Parish, LA	na
EP4D	30º36′0″N	87º54′36″W	Mobile Bay, AL	5.2
EP5I	30º27′0″N	88º6′36″W	Fowl River, AL	4.8
EP6D	30º24′36″N	88º54′0″W	Biloxi, MS	6.2
EP8D	31º33′36′ N	91º26′24″W	Mississippi River, LA	7.0

[z] Soil samples were collected where possible to determine soil pH at the collection site, na = not available.

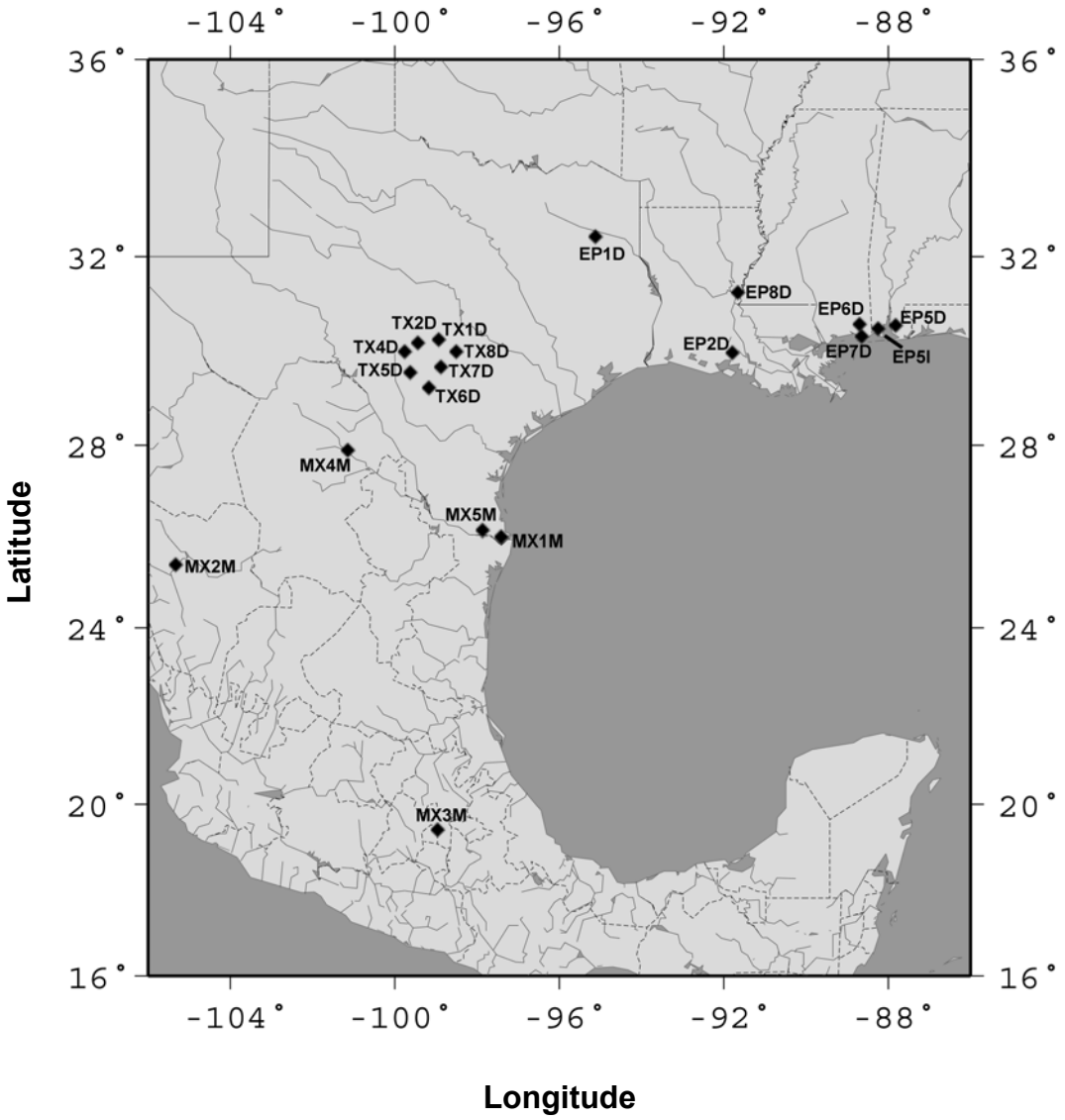

Figure 12. Locations of mother trees providing seeds for open-pollinated families of *Taxodium distichum* used in alkalinity tolerance screenings. Open-pollinated family identities are indicated beside the symbols.

Seedlings were planted in the field on 18 June 2004. Plants were arranged in a randomized complete block design with 13 families in 20 blocks containing 2 replications of each family per block. The field site was located at the Texas A&M Research and Extension Center at Dallas (USDA hardiness zone 8a/7b). The soil at the site is an Austin silty clay, 1 to 3 % slopes and has a pH of approximately 8.0. The trees were irrigated using T-Tape (T-Systems International, Inc., San Diego, Calif.) as needed and no additional fertilizer was added. Tree heights and trunk diameters were measured at the time of planting and again in Dec. 2004, 2005, and 2006. On 10 Aug. 2005, the seedlings were rated on the level of chlorosis they were exhibiting. The ratings were done on a four point scale: 1 – very chlorotic, 2 – slightly chlorotic, 3 – green, 4 – very green.

Growth indices for both height and trunk diameter were calculated as follows: growth index = (new measure – previous measure)/previous measure. This is analogous to relative growth rate calculations, except it is based on non-destructive measures rather than dry masses (Arnold et al., 2007).

Height growth indices and trunk diameter growth indices were analyzed using univariate analysis in the GLM procedure and Pearson's correlation analysis in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Chlorosis rating data was analyzed using Chi-square analysis and Spearman's rho analysis. Hierarchical cluster analysis in SPSS utilizing squared Euclidean distance and nearest-neighbor clustering

based on both growth indices and the chlorosis rating data was used to generate dendrograms.

Greenhouse Screening 1

Seven open-pollinated families of *Taxodium distichum* (Table 9, Fig. 12) collected in the late summer and fall of 2003 were grown in the nursery in 6.1 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) as described for plants used in the field studies, but were moved to a greenhouse on 6 June 2005 to begin controlled screenings for alkalinity tolerance. $KHCO_3$ (Sigma-Aldrich, Inc., St. Louis, Mo.) was used as an alkalinity source and was applied in the water at each irrigation. The treatment levels of $KHCO_3$ were 0, 4, 8, 12 mM. Mean EC and pH of irrigation solutions are reported in Table 10. Each container received 1000 mL of water per irrigation. This yielded approximately a 25% leaching fraction per irrigation. Plants were arranged in a randomized complete block design with seven open-pollinated families and two blocks containing three replications per block. Plants were grown in a greenhouse with 26.7ºC / 23.9ºC day/night temperature set points. Typical PAR, as measured in mid-afternoon on 30 June 2005, was 713 $\mu mol \cdot m^{-2} \cdot s^{-2}$. Height and diameter measurements were taken throughout the experiment and dry mass (14 d at 60ºC) of roots and shoots was measured at the end of the study. Leaf chlorophyll content was determined utilizing the acetone extraction methodology described by Harborne (1998). This was done at the initiation of the experiment and on 14 Aug. 2005, just before termination of the study. Leachate EC and pH was monitored periodically throughout the study. Additionally, a subjective evaluation of foliar chlorosis was taken

immediately before the study was harvested on 17 Aug. 2005. The ratings were assigned as described for the field screening study.

Height, truck diameter, and dry mass data were analyzed using univariate analysis in the GLM procedure in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Chlorosis and necrosis ratings were analyzed with loglinear analysis in SPSS. Leaf chlorophyll content was not analyzed because the second extraction failed.

Table 9. Localities of 7 mother trees providing seeds of open-pollinated families of *Taxodium distichum* collected in the late summer and fall of 2003 used in greenhouse screenings.

Family[z]	**Latitude**	**Longitude**	**Locale**	**pH**[y]
MX2M	25º18′36′N	104º38′24′W	Rio Nazas, MX	na
MX3M*	19º30′0′N	98º54′36′W	Bolleros, MX	na
MX4M*	27º51′0′N	101º7′48′W	Rio Sabinas, MX	na
TX4D*	29º43′12′N	99º45′0′W	Frio River, TX	7.6
TX6D*	29º0′36′N	98º34′48′W	Atascosa River, TX	7.1
EP7D	30º23′24′N	88º55′48′W	Biloxi, MS	5.6
EP8D*	31º33′36′N	91º26′24′W	Mississippi River, LA	7.0

[z] * indicates open-pollinated family was used in both greenhouse screenings.
[y] Soil samples were collected where possible to determine soil pH at the collection site, na = not available.

Table 10. Mean pH and electrical conductivity (EC) of irrigation solution containing four treatment levels of $KHCO_3$ applied to 6.1 L containers of *Taxodium distichum* seedlings and container leachate collected in 2005.

Level of $KHCO_3$	Solution (pH)	Leachate (pH)	Solution ($mS \cdot cm^{-1}$)	Leachate ($mS \cdot cm^{-1}$)
Control (0mM)	6.9 ± 0.1[z]	7.3 ± 0.1	0.03 ± 0.02	0.80 ± 0.1
Low (4mM)	8.0 ± 0.1	8.2 ± 0.1	0.45 ± 0.10	1.20 ± 0.1
Medium (8mM)	8.1 ± 0.1	8.5 ± 0.1	0.85 ± 0.13	1.20 ± 0.2
High (12mM)	8.2 ± 0.1	8.5 ± 0.1	1.25 ± 0.18	1.40 ± 0.3

[z] Values are means of 5 samples ± standard errors.

Greenhouse Screening 2

Five open-pollinated families of *Taxodium distichum* were selected for screening in the spring of 2006 (Table 9, Fig. 12). Families were selected to represent the ecophysiographic variation between the "Mexican" type populations (south Texas and Mexico localities) and those from central Texas because of the superior performance of genotypes from these regions in the initial screening. Cuttings from multiple trees per family from a stock block maintained in the field in College Station, Texas were rooted on 20 Mar. 2006. Cuttings were treated with a 8000 $mg \cdot L^{-1}$ IBA and 4000 $mg \cdot L^{-1}$ NAA dip (Dip 'n Grow, Inc., Clackamas, Ore.) and were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture, Bellevue, Wash.). Rooted cuttings were planted on 12 May 2006 into 2.5 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with calcined clay (Oil-Dri Corporation of America, Alpharetta, Ga.) amended with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ micronutrient fertilizer 0N-0P-0K-6Ca-3Mg-12S-17Fe (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), and

4.15 kg·m^{-3} $CaMgCO_3$ (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown in a greenhouse with 26.7ºC / 23.9ºC day/night temperature set points. Typical PAR, as measured in mid-afternoon on 30 Aug. 2006, was 702 µmol·m^{-2}·s^{-2}.

The study was initiated on 16 Aug. 2006. $KHCO_3$ (Sigma-Aldrich, Inc., St. Louis, Mo.) was used as an alkalinity source and was applied in the water at each irrigation. The treatment levels of $KHCO_3$ were 0, 4, 8, 12 mM. Irrigation also included 50 ppm N of from a 15N-2.2P-12.5K-5Ca-2Mg soluble fertilizer (Peters Excel®, Scotts Co., Marysville, Ohio). Mean EC and pH of irrigation solution is reported in Table 11. Each container received 500 mL of solution per irrigation. This yielded approximately a 25% leaching fraction per irrigation. Plants were arranged in a randomized complete block design with five open-pollinated families in three blocks and two replications of each family per block. Height and diameter measurements were taken at the initiation and termination of the experiment and dry mass of roots and shoots was measured at the end of the study. Leaf chlorophyll content was determined utilizing the acetone extraction methodology described by Harborne (1998) at the beginning of the study, but were not repeated at the end because no change in substrate pH was observed. Pooled sub-samples of the substrate were collected at the end of the study and EC and pH were measured using a 1:1 soil-water extraction method described by Richards (1969).

Height, trunk diameter, and dry mass data were analyzed using univariate analysis in the GLM procedure in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Chlorosis and necrosis ratings were analyzed with loglinear analysis in SPSS.

Table 11. Mean pH and electrical conductivity (EC) of irrigation solution containing four treatment levels of $KHCO_3$ applied to 2.5 L containers of *Taxodium distichum* cuttings and substrate water collected by 1:1 soil water extraction technique in 2006.

Level of $KHCO_3$	Solution (pH)	Leachate (pH)	Solution ($mS \cdot cm^{-1}$)	Leachate ($mS \cdot cm^{-1}$)
Control (0mM)	7.0 ± 0.1[z]	6.2 ± 0.1	0.02 ± 0.10	4.3 ± 0.1
Low (4mM)	7.6 ± 0.1	6.3 ± 0.1	0.36 ± 0.11	4.6 ± 0.1
Medium (8mM)	7.9 ± 0.1	6.3 ± 0.1	0.67 ± 0.13	4.4 ± 0.2
High (12mM)	8.0 ± 0.1	6.4 ± 0.2	1.30 ± 0.12	4.7 ± 0.1

[z] Values are means of 5 samples ± standard errors.

Results and Discussion

Field Screening

There was significant variation in the performance of open-pollinated families on the alkaline study site in Dallas. The Chi-square test for chlorosis rating and open-pollinated family was highly significant ($P \geq 0.0001$). The percentage of individuals in a given open-pollinated family receiving each rating category indicated that families from Mexico and south Texas, which are var. *mexicana*, generally had the most individuals with "greener" ratings (Fig. 13). Eastern families tended to have the most individuals with the most chlorotic ratings, and the central Texas families were intermediate. This pattern was expected based on the general trend of increasing soil pH from east to west in the U.S. (Table 8). Marcar et al. (2002) reported a similar trend in high pH tolerance of *Eucalyptus camaldulensis* provenances in Australia. They report that seedlings from localities with higher soil pH showed less growth reduction when grown in substrates with a pH of 9.5. A hierarchical cluster analysis based chlorosis ratings for each family

suggests the existence of three groups as well (Fig. 14). However, growth data divides the families into just two groups (Fig. 15 and 16). The trunk diameter growth index (R^2 = 0.795, $P \leq 0.001$) and height growth index (R^2 = 0.604, $P \leq 0.001$) differed significantly among families. The trunk diameter growth index was larger for the central and south Texas and Mexican families than those from more eastern sites (Fig. 15). The same pattern exists for the height growth index (Fig. 16). When chlorosis ratings and both growth indices are utilized in hierarchical cluster analysis, three clusters are still evident, although the central Texas families cluster more closely with the Mexican families than in analyses based on chlorosis alone (Fig. 17). The biggest change in this cluster analysis is the inclusion of family TX8D in the cluster of Mexican and south Texas families (Fig. 17). The noticeably "greener" ranking of family TX8D compared to other central Texas families (Fig. 13) likely accounts for this shift. A Spearman's rho correlation analysis indicates a strong relationship between open-pollinated family and chlorosis rating (R^2 = -0.753, $P \leq 0.001$), and chlorosis rating and both height growth index (R^2 = 0.526, $P \leq 0.001$) and trunk diameter growth index (R^2 = 0.598, $P \leq 0.001$). A Pearson's correlation analysis indicates a relationship between open-pollinated family and both height growth index (R^2 = -0.334, $P \leq 0.001$) and trunk diameter growth index (R^2 = -0.506, $P \leq 0.001$).

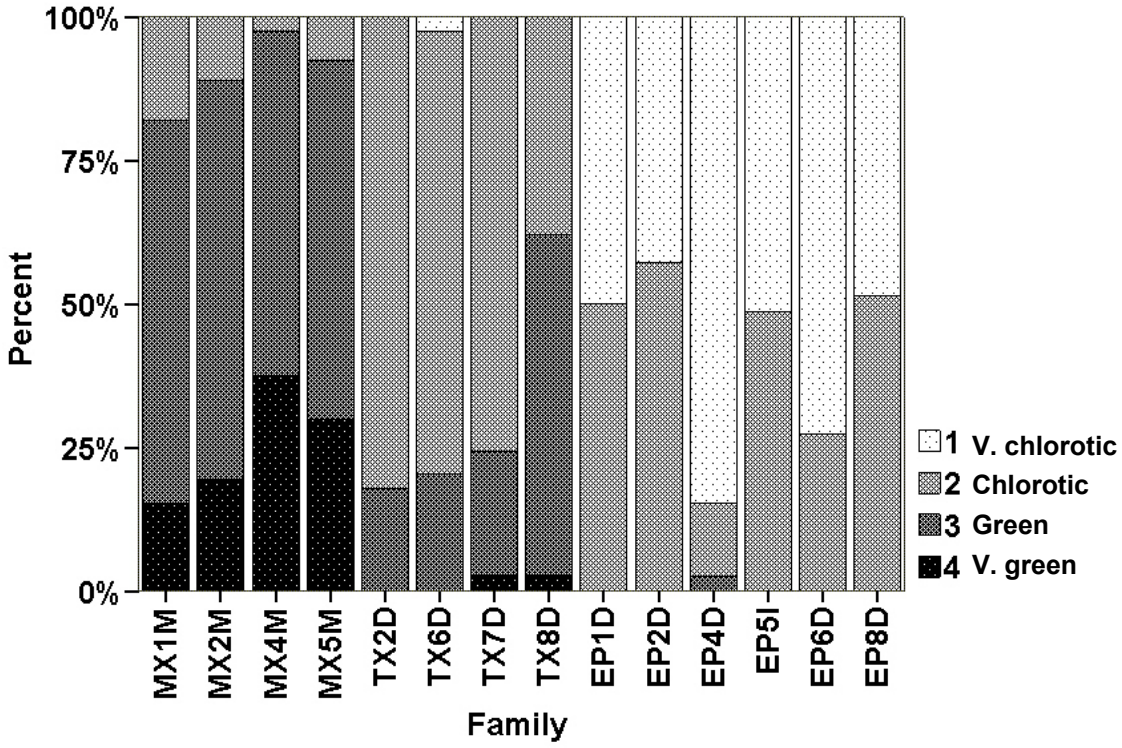

Figure 13. Percentage of 40 individuals in each of 14 open-pollinated families that received a given rank on a chlorosis rating scale of 1 to 4. The shade of the bar represents the relative shade of green of the plant.

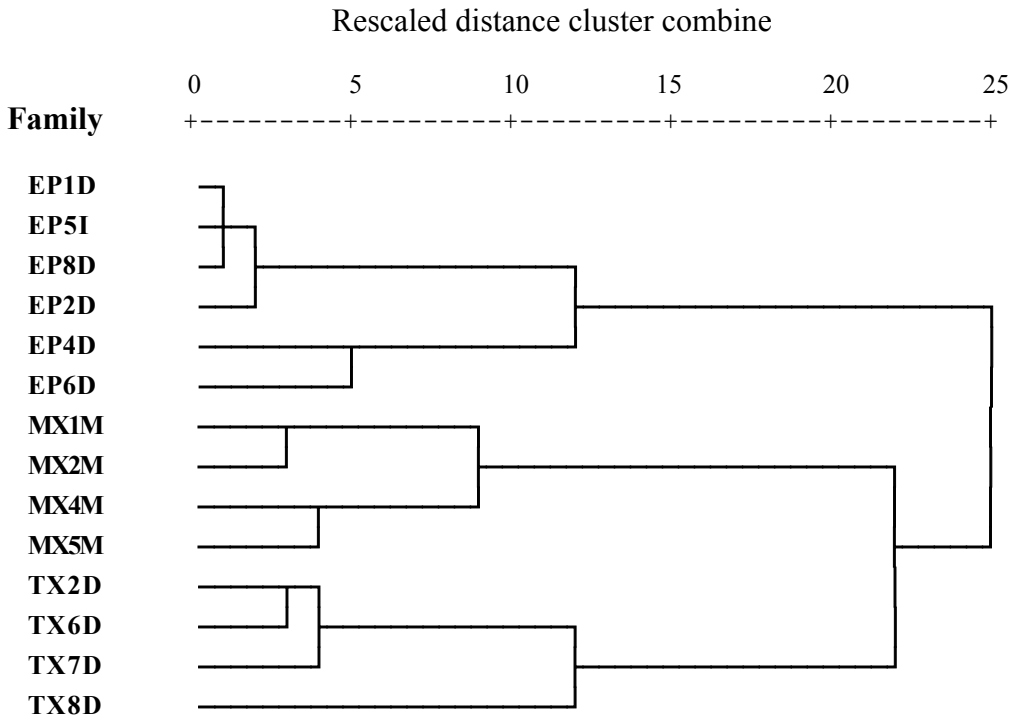

Figure 14. Hierarchical cluster analysis dendrogram using complete linkage based on chlorosis ratings of 14 open-pollinated families of *Taxodium distichum* on an alkaline site in Dallas, Texas.

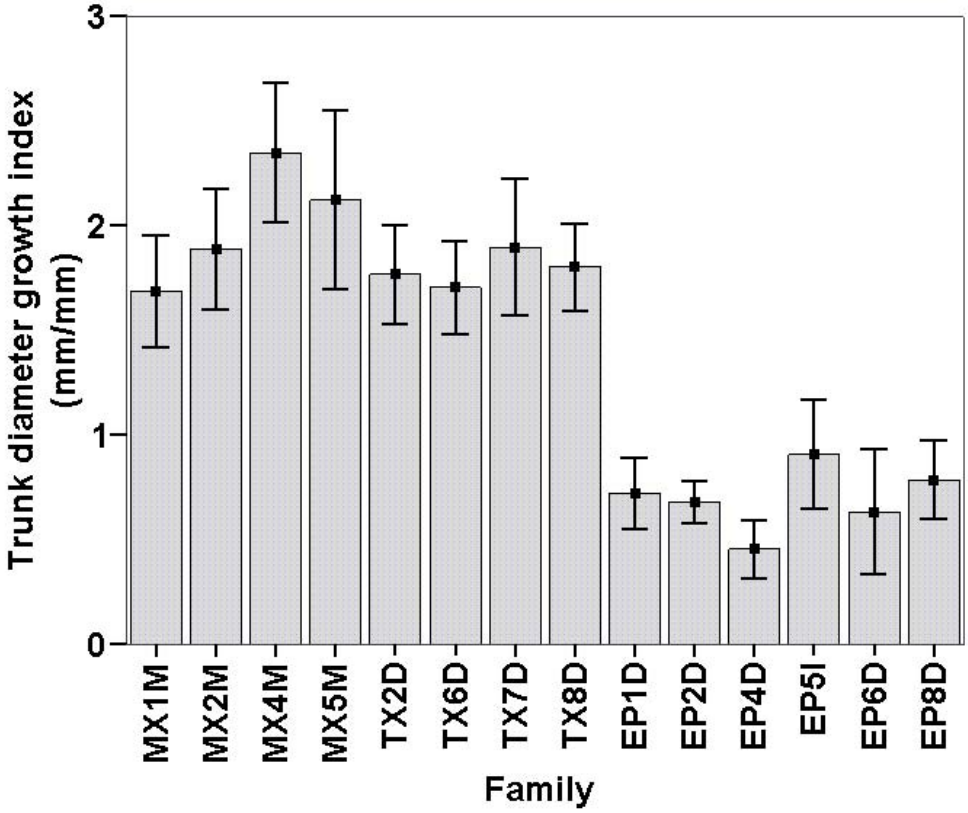

Figure 15. Trunk diameter growth indices of 14 open-pollinated families of *Taxodium distichum* on an alkaline site in Dallas, Texas. Values represent mean of 40 observations ± 95% confidence interval.

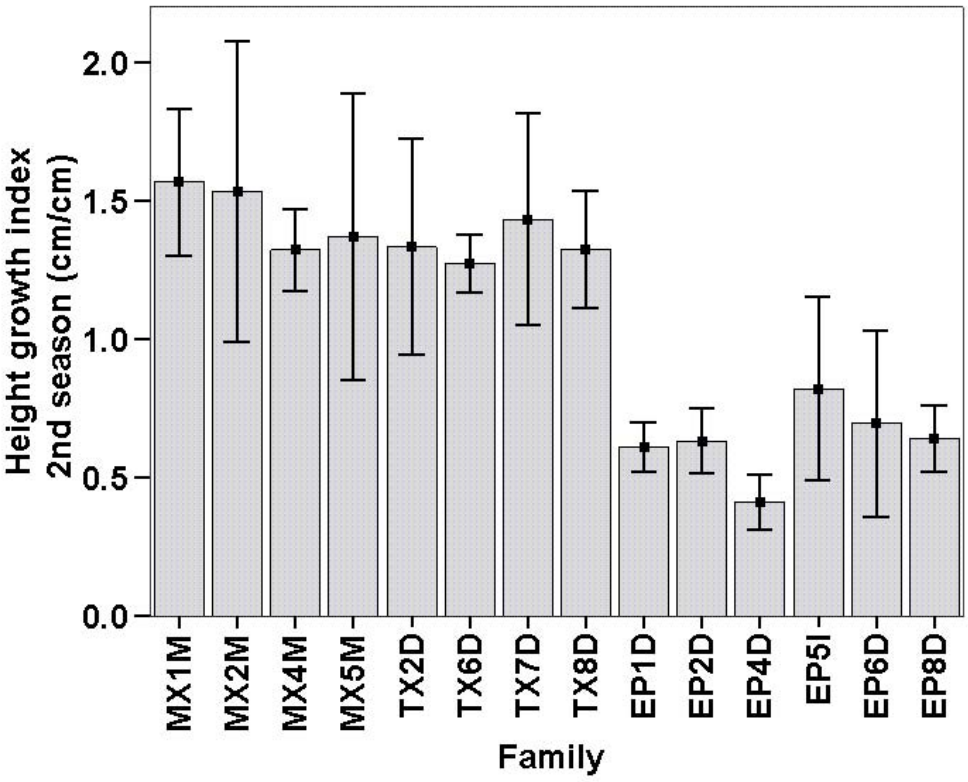

Figure 16. Height growth indices of 14 open-pollinated families of *Taxodium distichum* on an alkaline site in Dallas, Texas. Values represent mean of 40 observations ± 95% confidence interval.

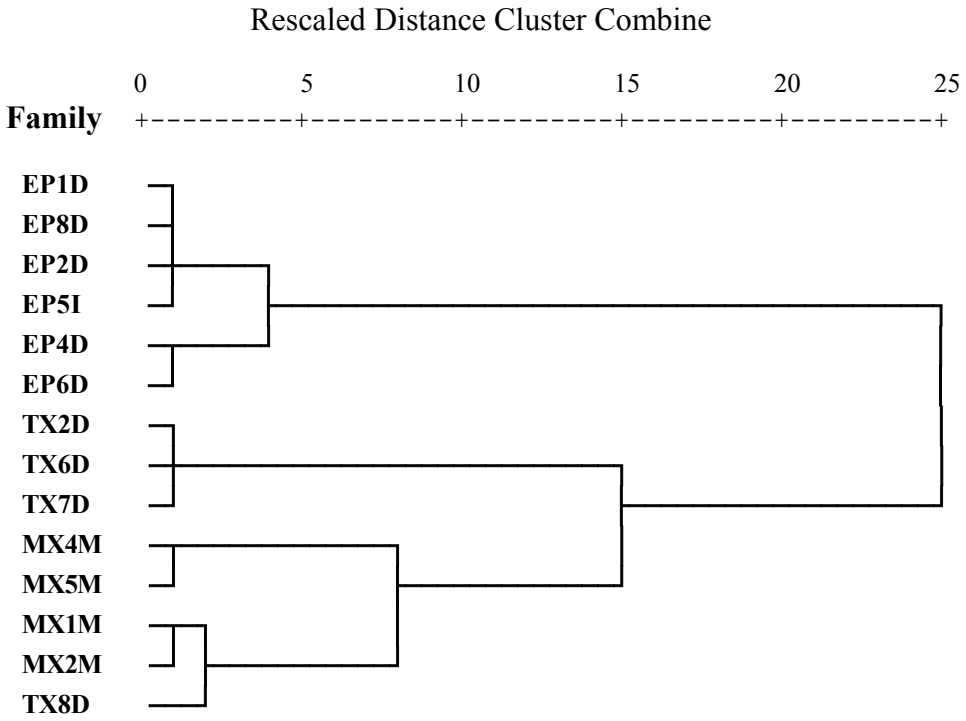

Figure 17. Hierarchical cluster analysis dendrogram using complete linkage based on chlorosis ratings and trunk diameter growth index and height growth index of 14 open-pollinated families of *Taxodium distichum* on an alkaline site in Dallas, Texas.

Greenhouse Screening 1

No treatment response ($P \leq 0.05$) was observed within open-pollinated families across $KHCO_3$ levels for growth data. Loglinear analysis of chlorosis ratings found no significant effect of within or among bicarbonate level and open-pollinated family ($P \geq 0.05$). Mean leachate pH and EC increased with increased levels of $KHCO_3$ (Table 10). The lack of response may have been due to the near ideal growing conditions under which the plants were cultivated.

Greenhouse Screening 2

No treatment response ($P \leq 0.05$) was observed within open-pollinated families across $KHCO_3$ levels. The 1:1 soil water extraction showed that the induced pH across the treatment levels was insufficiently high to induce growth reduction or chlorosis (Table 11).

The failure of both greenhouse screenings is puzzling and may be explained by several factors. The first is the better growing conditions present in a greenhouse environment. The second may be the duration of the studies. Valdez-Aguilar and Reed (2006) were able to induce a response in two cultivars of *Hibiscus rosa-sinensis* L. and Shi and Byrne (1995) were able to induce chlorosis in *Prunus* L. rootstocks in a similar time period and under similar conditions. It seems likely that it may take longer to induce alkalinity effects on this species than more rapidly growing and/or herbaceous species. It may be that the nutrient source also plays a role in the onset of alkalinity effects. The micronutrients, especially Fe, are available to the plant in a different form in commercial fertilizers than those commonly found in field soil (Marschner, 1995).

The field study provided a reliable screening technique. It clearly shows that there is a geographic component to alkalinity tolerance in *Taxodium distichum*. Interestingly, Wood et al. (1998) found a similar geographic pattern for Zn deficiency in pecan [*Carya illinoinensis* (Wangenh.) K.Koch] provenances. They found that Texas and Mexican provenances tended to cluster together, separate from sources north and east of Texas. Their results were surprising because it indicated that provenances from areas with more alkaline soils (Mexico and Texas) showed higher levels of Zn deficiency. They attributed this to differential growth rates, indicating that the faster growth of the Texas and Mexican provenances was causal rather than physiological use or uptake efficiency of Zn.

Taxonomic ranking also seems to correlate with performance on an alkaline site. This is to be expected, as varietal status in this species has a strong geographic component (Denny and Arnold, 2007). Genotypes from south Texas and Mexico all belong to var. *mexicana*, montezuma cypress. All of the genotypes in central Texas are var. *distichum*, baldcypress. The vast majority of the sampled eastern genotypes are also var. *distichum*, the exception being family EP5I from the Fowl River in Alabama. This family was var. *imbricarium*, pondcypress. Montezuma cypress seems to be more tolerant of an alkaline site than the other varieties. If this variety is excluded, and var. *distichum* is considered alone, there is still a strong geographic component to the variation in tolerance of alkaline soils.

Care should be taken to select genotypes from regions shown to yield tolerant individuals. When selecting plant material for an alkaline sites, genotypes from Mexico

and south Texas should be preferred, followed by central Texas genotypes. The probable greater cold hardiness of genotypes from central Texas based on their more northern latitudes may dictate their use in preference over the south Texas and Mexican genotypes on alkaline sites in colder regions.

CHAPTER VI

EVALUATION OF SALINITY TOLERANCE OF SELECTED PROVENANCES OF *TAXODIUM*

Thirty three percent of irrigated land worldwide is affected by salinity (Marschner, 1995). Saline soils are especially abundant in the arid and semiarid regions of the world, usually because the rainfall is insufficient for proper leaching (Marschner, 1995). However, salt may be added to soils by rain and sea spray in some regions, especially coastal areas, and by road salt in others. Irrigation water may have 100-1000 $g \cdot m^{-3}$ of salt, and with an average irrigation application of 10,000 $m^3 \cdot ha^{-1} \cdot year^{-1}$, 907 to 9072 kg (1-10 tons) of salt can be added to the soil (Marschner, 1995). Sodium chloride is usually the dominant salt in problem areas, although other salts may be abundant as well, depending on the water source and the solubility of the salt (Marschner, 1995).

There are three ways that salinity restricts plant growth. First, it can induce water deficit. This is because the salts decrease the water potential of the rooting substrate which limits the ability of the plant to absorb water (Marschner, 1995; Wahome et al., 2001). Second, the plant can experience ion toxicity because of excess uptake of certain ions, most commonly Na^+ and Cl^- (Marschner, 1995; Wahome et al., 2001). Lastly, salt stress can cause an ion/nutrient imbalance in the plant. This is usually due to a decrease in the plants ability to absorb certain nutrients and to transport nutrients internally (Marschner, 1995; Wahome et al., 2001), often as a result of competiton with Na^+ or other cations.

Wahome et al. (2001) define salt tolerance as "the ability of a plant to maintain growth and metabolism under saline conditions". There are two mechanisms that a plant can use to adapt to a saline substrate: salt exclusion and salt inclusion (Marschner, 1995). Salt exclusion is by far the more common strategy of salinity tolerance among non-halophytes, and requires the plant to be able to avoid internal water deficit (Marschner, 1995). The plant's ability to prevent translocation of ions, especially Na^+, from the roots to the shoots is very important for excluders, because the exclusion of ions from the cell is not usually sufficient (Wahome et al., 2001). Inclusion usually requires a tolerance of high levels of ions, especially Na^+ and Cl^-, in the cytoplasm (Marschner, 1995), but may also involve sequestration of ions in cellular compartments. This strategy is normally limited to halophytes.

Classification of salt tolerance in plants is commonly based on a threshold electrical conductivity (EC) above which plant growth and development is impaired and on the rate of the decrease in growth after the threshold EC has been reached (Marschner, 1995). Large differences in intraspecific salt tolerance have been observed, as well as differences between ontogenetic stages of individuals (Marschner, 1995).

The purpose of this study is to determine if there is a geographic basis for salinity tolerance in *Taxodium* and to evaluate provenances in an effort to select those which yield individuals that are most adaptable/tolerant to these environmental stresses.

Materials and Methods

Open-pollinated family identity was coded with four alphanumeric characters. The first two letters signify the general geographic origin of the mother tree. 'MX'

signifies south Texas and Mexico, 'TX' signifies central Texas, and 'EP' denotes the southeastern U.S. The numeral is unique to an open-pollinated family from a given geographic area. The final letter indicates the taxonomic variety. 'M' indicates that the open-pollinated family belongs to the variety *mexicanum*, 'D' indicates var. *distichum*, and 'I' indicates var. *imbricarium*.

Substrate Salt Screening

Five open-pollinated families of *Taxodium distichum* were selected for screening in the spring of 2006 (Table 12 and Fig. 18). Families were selected to represent the ecophysiographic variation between the "Mexican" type populations and those from central Texas because of the superior performance of genotypes from these regions in initial screenings. Cuttings from multiple trees per open-pollinated family from a stock block maintained in the field in College Station, Texas were rooted on 20 March 2006. Cuttings were treated with a 8000 $mg \cdot L^{-1}$ IBA and 4000 $mg \cdot L^{-1}$ NAA dip (Dip 'n Grow, Inc., Clackamas, Ore.) and were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture, Bellevue, Wash.). Flats were placed under an intermittent mist (15 sec. dawn to dusk). Rooted cuttings were transplanted on 12 May 2006 into 2.5 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with calcined clay (Oil-Dri Corp. of America, Alpharetta, Ga.) amended with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ 0N-0P-0K-6Ca-3Mg-12S-17Fe micronutrient fertilizer (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), and 4.15 $kg \cdot m^{-3}$ $CaMgCO_3$ (Oldcastle Stone Products,

Thomasville, Pa.). Plants were grown in a greenhouse with 26.7 ºC day / 23.9 ºC night temperature set points. Typical PAR, as measured in mid afternoon on 30 August 2006, was 702 $\mu mol \cdot m^{-2} \cdot s^{-2}$.

Table 12. Localities of mother trees of open-pollinated families of *Taxodium distichum* used in salinity tolerance screening studies in 2006.

Family	**Screening**[z]	**Latitude**	**Longitude**	**Locale**
MX3M	S,F	19º30′0″N	98º54′36″W	Bolleros, MX
MX4M	S,F	27º51′0″N	101º7′48″W	Rio Sabinas, MX
TX6D	S,F	29º0′36″N	98º34′48″W	Atascosa River, TX
EP1D	F	32º20′24″N	94º42′0″W	Lake Cherokee, TX
EP2D	S	29º48′0″N	91º47′24″W	Iberia Parish, LA
EP4D	F	30º36′0″N	87º54′36″W	Mobile Bay, AL
EP6D	S	30º24′36″N	88º54′0″W	Biloxi, MS

[z] S=substrate salinity screening, F=foliar salinity screening

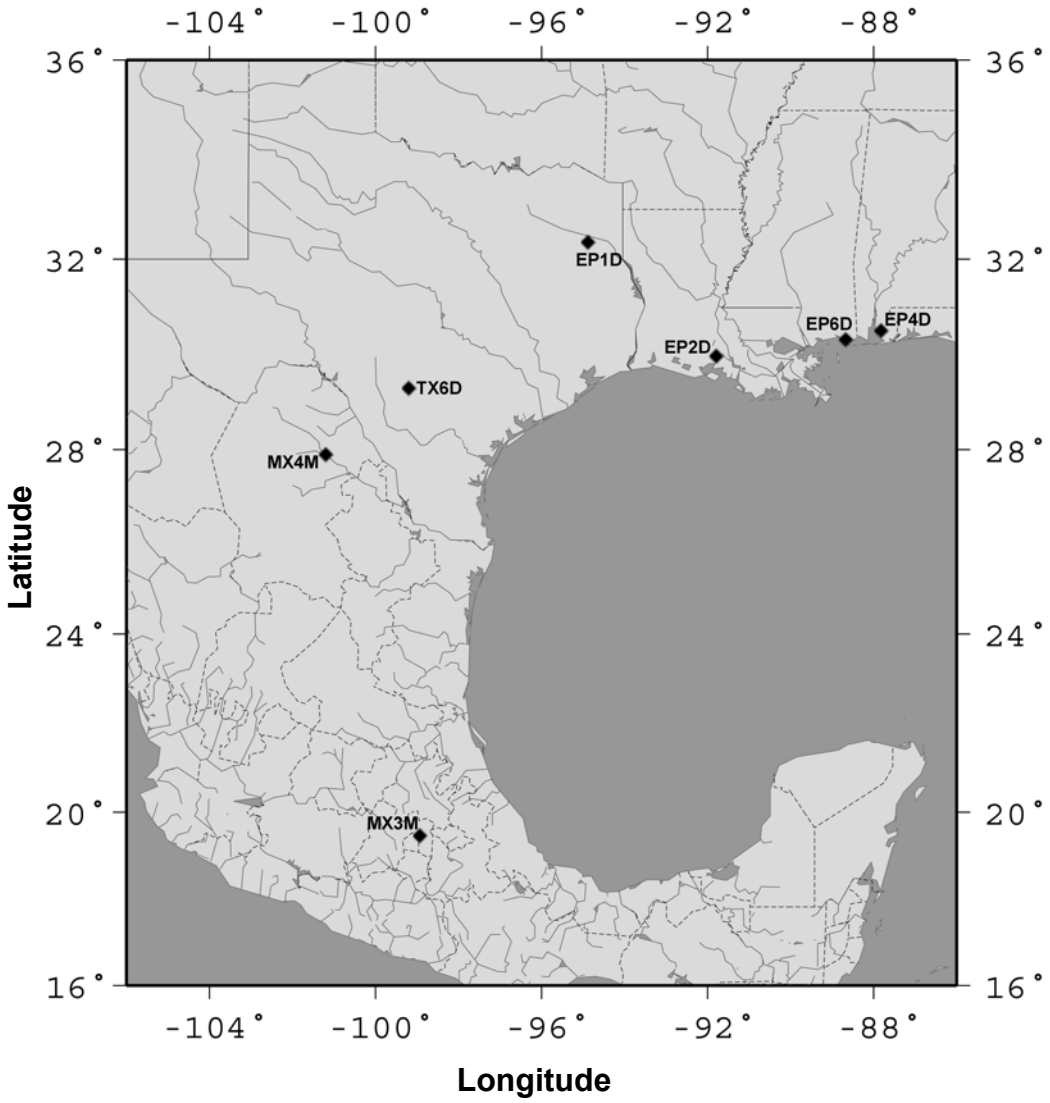

Figure 18. Localities of mother trees of open-pollinated families of *Taxodium distichum* used in salinity tolerance screening studies in 2006. Family identities are indicated beside the location symbols.

The study was initiated on 21 August 2006. NaCl (Mallinckrodt Chemicals, Phillipsburg, N.J.) and $CaCl_2$ (EM Science, Gibbstown, N.J.) were used in a ratio of 2:1 as a salinity source and was applied in the water at each irrigation. Treatment levels are based on Miyamoto et al. (2004) and included a control with no 2NaCl:1$CaCl_2$, a low 2NaCl:1$CaCl_2$ concentration (2000 $mg \cdot L^{-1}$), an intermediate 2NaCl:1$CaCl_2$ concentration (4000 $mg \cdot L^{-1}$), and a high 2NaCl:1$CaCl_2$ concentration (8000 $mg \cdot L^{-1}$). This produced irrigation solutions with the following electrical conductivities: 0.2 $mS \cdot cm^{-1}$, 3.9 $mS \cdot cm^{-1}$, 6.9 $mS \cdot cm^{-1}$, and 12.0 $mS \cdot cm^{-1}$, respectively. Irrigation also included 50 ppm N from a 15N-2.2P-12.5K-5Ca-2Mg soluble fertilizer (Peters Excel®, Scotts Co., Marysville, Ohio). The water source for the solutions was treated with reverse osmosis to remove background salinity. Each container received 500 mL of solution per irrigation. This yielded approximately a 25% leaching fraction per irrigation.

Plants were arranged in a randomized complete block design with three blocks containing two replicates in each block per family per treatment. Height and trunk diameter measurements were taken at the beginning and end of the study. Shoot fresh mass and shoot and root dry masses were measured at the end of the study. At harvest pre-dawn xylem water potentials were measured using a pressure chamber (Model 610, PMS Instrument Company, Albany, Ore.). Foliar damage ratings were assigned on a scale of 0 to 5 based on percent of the foliage showing damage. Ratings were assigned as follows: 0 - no damage, 1 – 1 to 25%, 2 – 26 to 50%, 3 – 51 to 75%, 4 – 76 to 99%, 5 – total foliar death (Fig. 19). Samples of the substrate were collected at the end of the

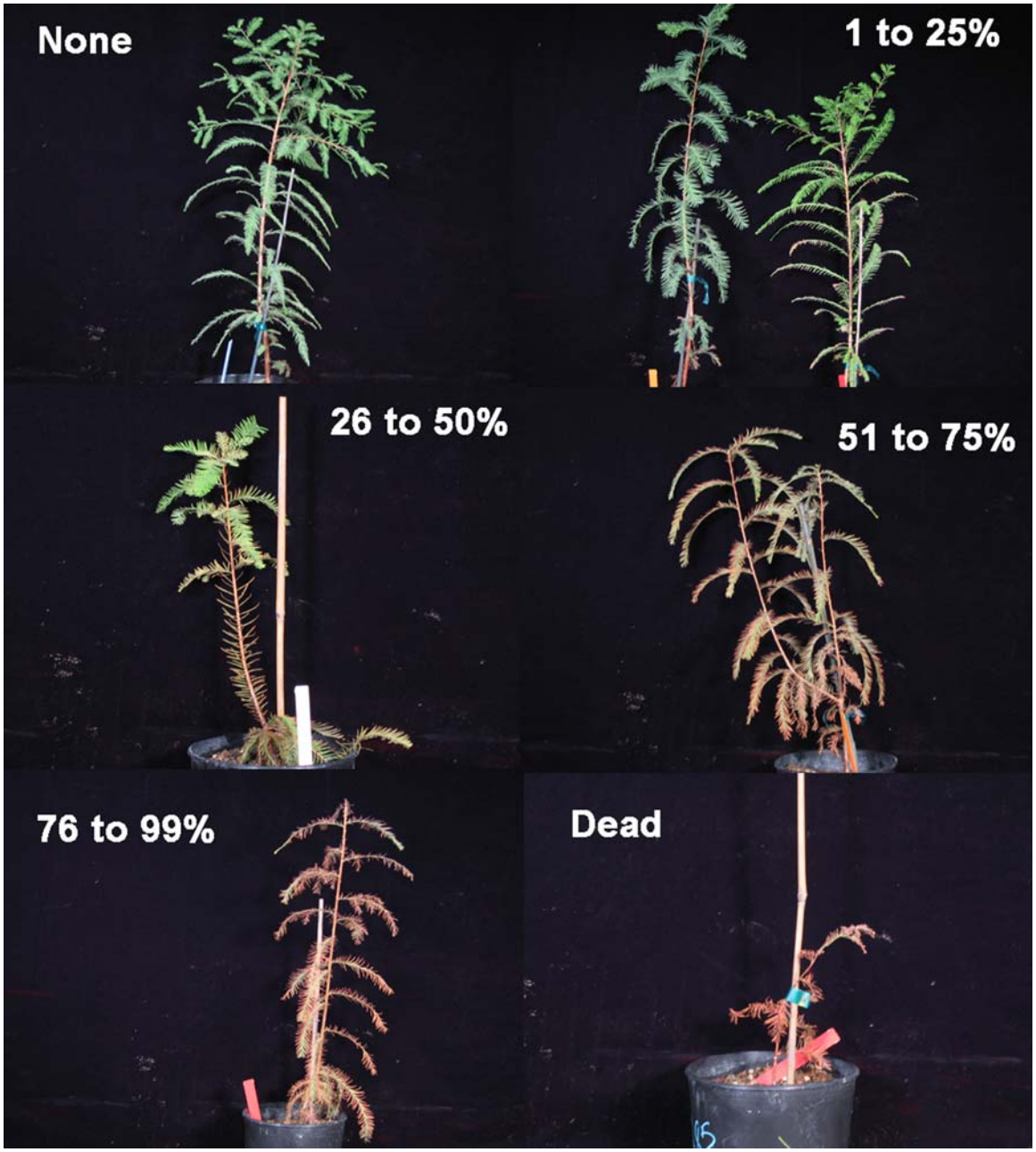

Figure 19. Examples of plants receiving each level of the foliar damage rating scale used in both screenings.

study and EC and pH were measure using a 1:1 soil-water extraction method described by Richards (1969). Height, trunk diameter, shoot and root dry mass, root to shoot ratio, and pre-dawn xylem water potential data were analyzed using univariate analysis in the GLM procedure in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Blocking effects were found to be non-significant ($P \geq 0.05$) and data was subsequently pooled accordingly. Foliar damage ratings were analyzed with Kruskal-Wallis analysis in the non-parametric procedure in SPSS. Survival data were analyzed with Chi-square analysis in SPSS.

Foliar Salt Screening

Five open-pollinated families of *Taxodium distichum* were selected for screening for foliar salt tolerance in the spring of 2006 (Table 12 and Fig. 18). Experimental plants were produced under the same conditions as were described for substrate screening trials with the following exceptions. The study was initiated on 18 Sept. 2006. NaCl (Mallinckrodt Chemicals, Phillipsburg, N.J.) was used as a salinity source and was applied as a foliar spray at each irrigation. Foliar applied treatments are based on Miyamoto et al. (2004) and included a control with no NaCl, a low NaCl concentration (800 $mg \cdot L^{-1}$), an intermediate NaCl concentration (1260 $mg \cdot L^{-1}$), and a high NaCl concentration (1850 $mg \cdot L^{-1}$). This produced irrigation solutions with the following electrical conductivities: 0.2 $mS \cdot cm^{-1}$, 1.6 $mS \cdot cm^{-1}$, 2.6 $mS \cdot cm^{-1}$, and 3.7 $mS \cdot cm^{-1}$, respectively. The water source for the solutions was treated with reverse osmosis. Following foliar applications, the substrate in the containers was immediately irrigated with a container volume of reverse osmosis treated water to leach any salts dropping on

the substrate surface to ensure that observed salinity responses were due primarily to foliar absorption.

Plants were arranged in a randomized complete block design with three blocks containing two replicates per family per treatment. Height and trunk diameter measurements were taken at the beginning and end of the study. Shoot fresh mass and shoot and root dry masses were measured at the end of the study. At harvest, pre-dawn xylem water potential was measured using a pressure chamber (Model 610, PMS Instrument Company, Albany, Ore). Foliar damage ratings were assigned as described for the substrate screening (Fig. 19). Samples of the substrate were collected at the end of the study and EC and pH were measure using a 1:1 soil-water extraction method described by Richards (1969). Height, trunk diameter, shoot dry mass, root to shoot ratio, pre-dawn xylem water potential, and shoot water content data were analyzed using univariate analysis in the GLM procedure in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Blocking effects were found to be non-significant ($P \geq 0.05$) and data was subsequently pooled accordingly. Foliar damage ratings were analyzed with Kruskal-Wallis analysis in the non-parametric procedure in SPSS.

Results and Discussion

Substrate Salt Screening

Chi-square analysis showed that open-pollinated families differed significantly in plant survival at both medium (4000 $mg \cdot L^{-1}$) and high (8000 $mg \cdot L^{-1}$) salinity treatments ($P \leq 0.002$) (Fig. 20). At the medium (4000 $mg \cdot L^{-1}$) salinity level, families MX3M, MX4M and EP2D had 100% survival, family EP6D had 0% survival, and family TX6D

had 66.7% survival (Fig. 20). Families EP6D and TX6D had 0% survival at the high (8000 mg·L^{-1}) salinity level, while family EP2D had 100% survival, family MX3M had 75% survival, and family MX4M had 83.3% survival (Fig. 20). Root to shoot ratio, shoot dry mass, height growth and trunk diameter growth did not differ significantly among open-pollinated families within salinity level treatments ($P \geq 0.05$) for substrate treatments (Table 13). Foliar damage ratings of families were significantly affected by salinity levels ($P \leq 0.01$) (Table 14). Ratings for families TX6D and EP6D deteriorated the most with increasing salinity (Fig. 21). Families MX3M and MX4M had the least damage at the low (2000 mg·L^{-1}) salinity level, followed by family TX6D, then family EP2D, and family EP6D, which had the worst ratings (Fig. 21). At the medium (4000 mg·L^{-1}) salinity level, family MX3M had the least foliar damage, followed by families MX4M and EP2D, then family TX6D, and lastly, family EP6D with total foliar death (Fig. 21). Family EP2D had the least foliar damage at the high (8000 mg·L^{-1}) salinity level, followed by families MX3M and MX4M, while families TX6D and EP6D had total foliar death (Fig. 21). Pre-dawn xylem water potential differed significantly among families within salinity levels ($P \leq 0.001$) (Table 13). None of the open-pollinated families were experiencing substantial water stress (xylem water potential > -1.5 MPa) at the low (2000 mg·L^{-1}) salinity level (Fig. 22). However, families TX6D and EP6D were both under substantial water deficits at the medium (4000 mg·L^{-1}) and high (8000 mg·L^{-1}) salinity levels (Fig. 22). Families MX3M and MX4M exhibited only mild water stress at the medium (4000 mg·L^{-1}) salinity level, but dropped below -1.5 MPa xylem water

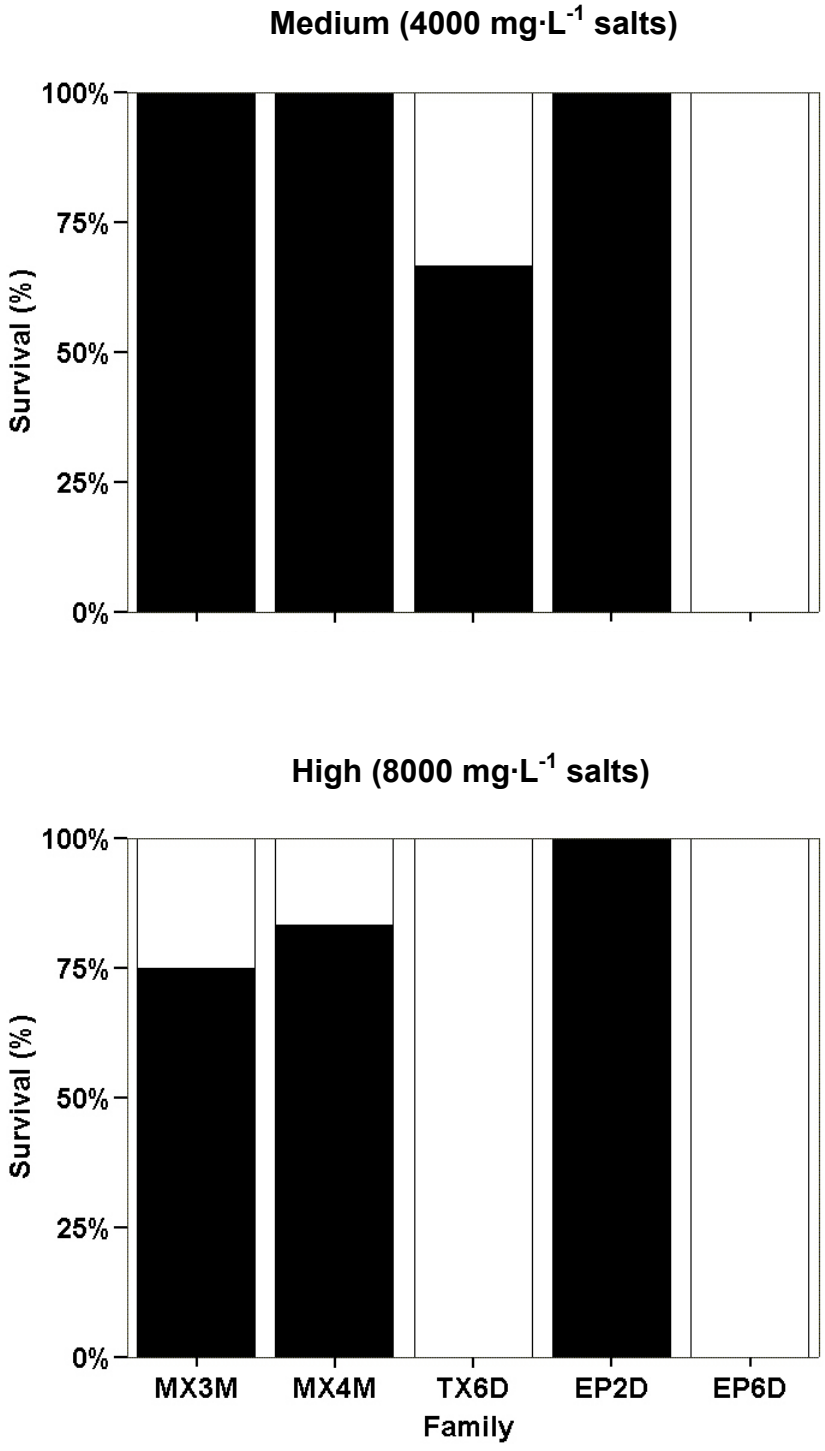

Figure 20. Percent survival for open-pollinated families of *Taxodium distichum* at moderate (top) and high (bottom) levels of substrate salinity. n=6.

Table 13. ANOVA table for root to shoot ratio, shoot dry mass, height growth, trunk diameter growth, and pre-dawn xylem water potential for five open-pollinated families of *Taxodium distichum* used in substrate salt tolerance screening.

	Root : Shoot Ratio	Shoot Dry Mass	Height Growth	Trunk Diameter Growth	Pre-dawn Xylem Water Potential
Corrected Model	0.001[z]	<0.001	0.338	0.075	<0.001
Intercept	<0.001	<0.001	0.215	<0.001	<0.001
Family	<0.001	<0.001	0.936	0.581	<0.001
Level	0.427	0.003	0.050	0.001	<0.001
Family X Level	0.567	0.085	0.313	0.898	<0.001

[z] Significance of this component in the statistical model.

Table 14. Kruskal-Wallis test for foliar damage ratings at each salinity level for five open-pollinated families of *Taxodium distichum* used in substrate salt tolerance screening.

	Mean Rank			
Family	Control (0 mg·L^{-1})	Low (2000 mg·L^{-1})	Medium (4000 mg·L^{-1})	High (8000 mg·L^{-1})
MX3M	12.500	7.250	4.375	7.625
MX4M	12.500	6.000	8.500	9.417
TX6D	12.500	13.333	17.500	20.000
EP2D	12.500	18.333	12.100	9.667
EP6D	12.500	22.500	22.000	20.000
Sig.	>0.999	0.002	0.003	0.006

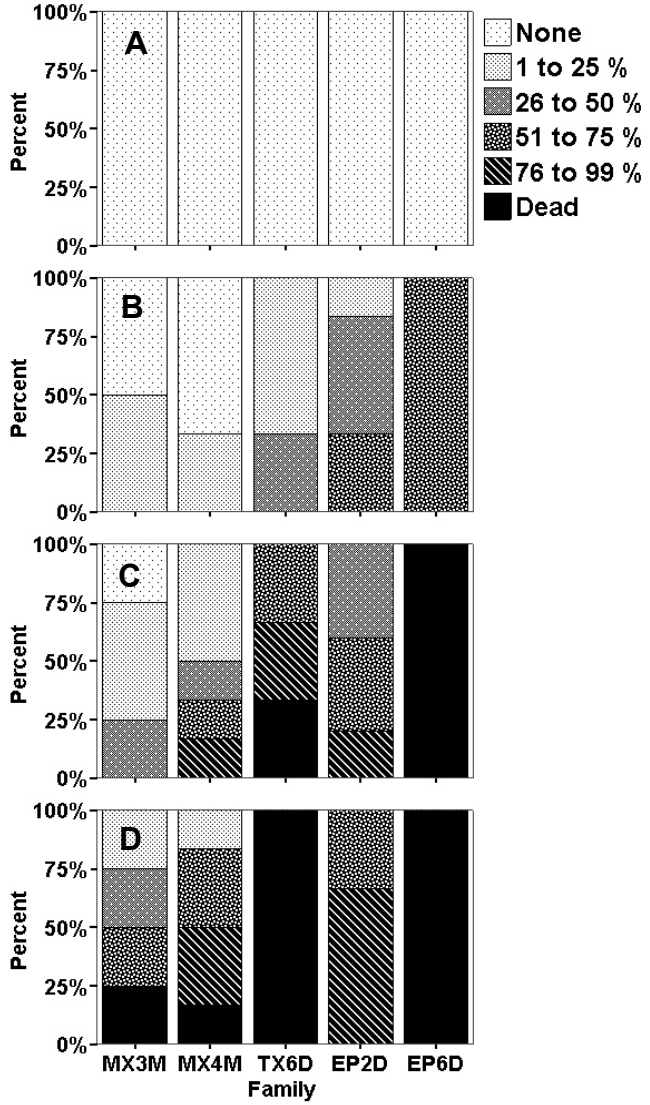

Figure 21. Foliar damage ratings of 5 open-pollinated families of *Taxodium distichum* at four levels of substrate salinity. The four levels were 0 $mg \cdot L^{-1}$ (A), 2000 $mg \cdot L^{-1}$(B), 4000 $mg \cdot L^{-1}$(C), 8000 $mg \cdot L^{-1}$(D). The various shading of portions of each bar represents the percentage of individuals in a family at each salinity level receiving a certain rating. n = 6.

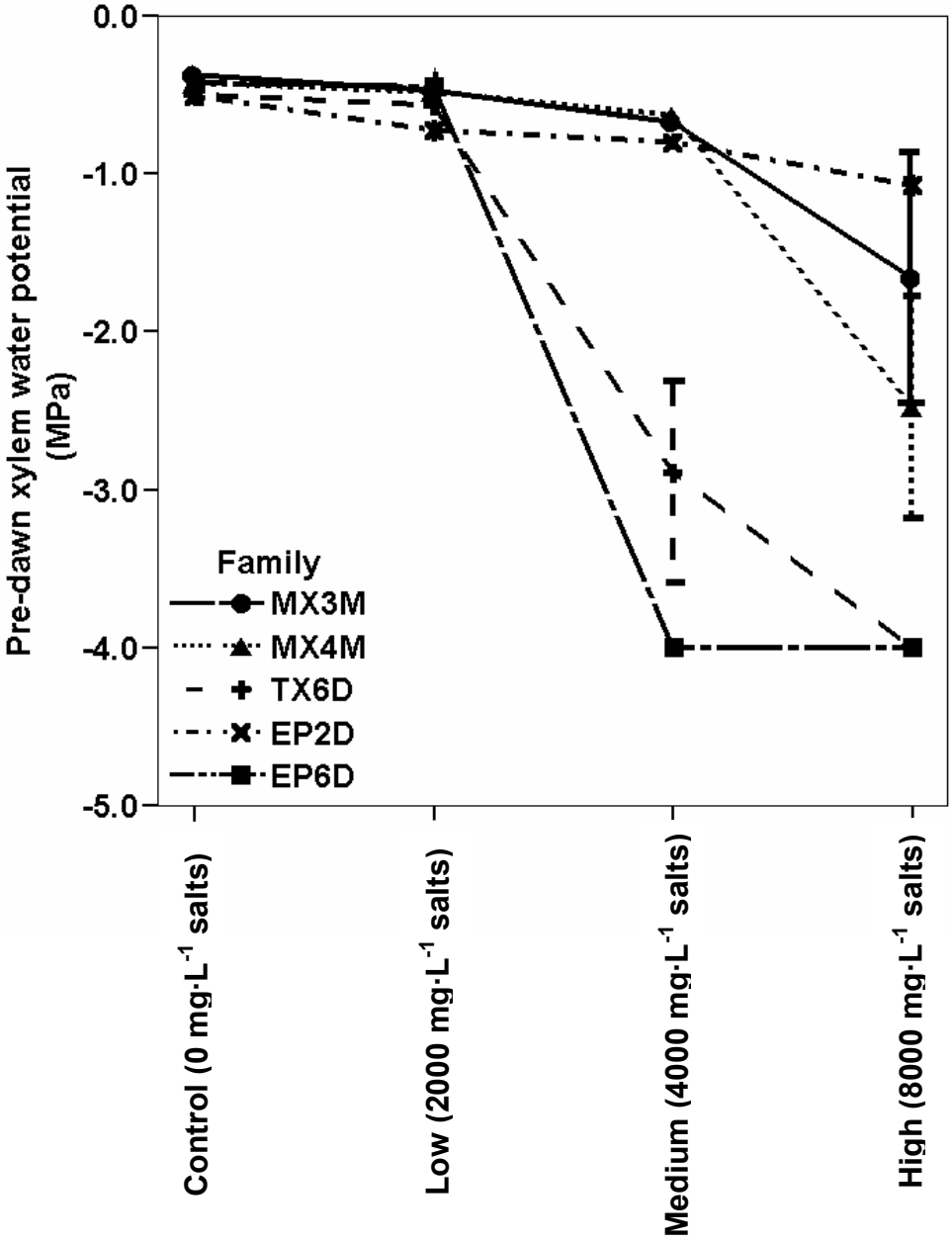

Figure 22. Pre-dawn xylem water potential for five open-pollinated families of *Taxodium distichum* at four levels of substrate salinity. Symbols represent means ± standard error of 6 observations. Symbols obscure the error bars that are not apparent.

potential at the high (8000 $mg \cdot L^{-1}$) salinity level (Fig. 22). Family EP2D did not drop below -1.0 MPa xylem water potential at any salinity level (Fig. 22).

All of the open-pollinated families were relatively tolerant of low levels of substrate salinities. Niknam and McComb (2000) state that at moderate levels of salinity, salt exclusion is the main adaptive strategy and tolerance of high ion concentrations is important at high levels of salinity. There is also the osmotic component to salinity stress to be considered. The increased tolerance of families MX3M, MX4M and EP2D at the higher levels is likely due to a combination of both osmotic and ionic stress tolerance. The increased osmotic stress avoidance is observed in the less negative pre-dawn xylem water potentials of the more tolerant families at moderate and high levels of salinity (Fig. 22). The relatively greater tolerance to water stress for Mexican families was observed in the drought tolerance screenings of open-pollinated families (see Chapter IV). It was not observed in family EP2D. This implies that there is a different stress tolerance mechanism for the osmotic component to salinity stress than those stress tolerance mechanisms utilized to cope with drought stress.

The performance of family EP2D at high levels of substrate salinity is somewhat surprising because it comes from an inland site in Louisiana. The poor performance of family EP6D, from Mobile Bay, is also surprising. Allen et al. (1994) report that the most salinity tolerant open-pollinated families of *Taxodium* come from brackish sources, citing greater biomass production, survival, height, and leaf area. Krauss et al. (1998) also report greater seed germination capacity among open-pollinated families of *Taxodium* from brackish sites. However, Pezeshki et al. (1995) reported that *Taxodium*

seedlings from freshwater sources outperformed families from brackish sources. Krauss et al. (1999) reported greater root growth from a single fresh water source family compared to brackish source families. These reports suggest that it is possible to find genotypes from non-saline/non-brackish sources which exhibit enhanced salinity tolerance. Family EP2D may be another example of this phenomenon.

The better performance of the Mexican families, both *T. distichum* var. *mexicana*, at higher salinity levels suggests that there may be some taxonomic level differences in salinity tolerance as well.

Foliar Salt Screening

Plant height, trunk diameter, shoot dry mass, root to shoot ratio, pre-dawn xylem water potential and shoot water content of open-pollinated families were all unaffected by foliar salt treatments ($P \geq 0.05$) (Table 15). Devitt et al. (2003) also found that foliar applied salts did not result in reduced growth nor affect water status. Only foliar damage ratings differed among open-pollinated families and among treatment levels (Table 16). There were no differences among families at the control level, which had no foliar damage evident (Table 16 and Fig. 23). At all of the treatment levels, families MX3M and MX4M ranked the best, having the least foliar salt damage (Table 16 and Fig. 23). Families EP1D and EP4D ranked the lowest at all treatment levels and family TX6D was intermediate (Table 16 and Fig. 23).

In general, open-pollinated families from Mexico were more tolerant of salt spray than families from the southeastern U.S. The family from central Texas (TX6D) was intermediate in tolerance. Although there seems to be a geographic pattern to the

tolerance differences observed, the difference may be due to the differential leaf wetting times. No data was taken on amount of time the leaves remained wet, but it was observed that the Mexican families (MX3M and MX4M) tended to be harder to wet and dried the fastest, followed by family TX6D, then families EP1D and EP4D. This difference may be attributable to leaf surface differences. The more western provenances are waxier in appearance and may have a thicker cuticle. Devitt et al. (2003) found that the longer wet periods of foliage resulted in lower Ca concentrations and higher Na concentrations in the leaves. This would explain the response pattern observed in this study. The poor performance of family EP4D is somewhat surprising because of its origin on Mobile Bay in Alabama. The mother tree was growing in close proximity to the beach. This family was suspected to have increased tolerance to foliar salts due to its natural exposure to salt spray. It did show a slight improvement over the other "eastern-type" family (EP1D), but not in comparison to more western provenances.

Table 15. ANOVA for plant height, trunk diameter, root to shoot ratio, shoot dry mass, pre-dawn xylem water potential and shoot water content for five open-pollinated families of *Taxodium distichum* used in foliar salt tolerance screening.

	Height	Trunk Diameter	Shoot Dry Mass	Root:Shoot Ratio	Pre-dawn Xylem Water Potential	Shoot Water Content
Corrected Model	<0.001[z]	0.002	<0.001	<0.001	<0.001	<0.001
Intercept	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Family	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Level	0.836	0.815	0.406	0.120	0.038	0.729
Family X Level	0.982	0.483	0.357	0.393	0.195	0.069

[z] Significance of this component in the statistical model.

Table 16. Kruskal-Wallis test for foliar damage ratings at each salinity level for five open-pollinated families of *Taxodium distichum* used in foliar salt tolerance screening.

	Mean Rank			
Family	Control (0 $mg \cdot L^{-1}$)	Low (800 $mg \cdot L^{-1}$)	Medium (1260 $mg \cdot L^{-1}$)	High (1850 $mg \cdot L^{-1}$)
MX3M	15.500	6.917	9.000	7.000
MX4M	15.500	8.333	9.000	9.333
TX6D	15.500	18.167	12.083	12.167
EP1D	15.500	20.625	23.500	24.000
EP4D	15.500	20.500	22.833	23.000
Sig.	>0.999	0.003	0.001	<0.001

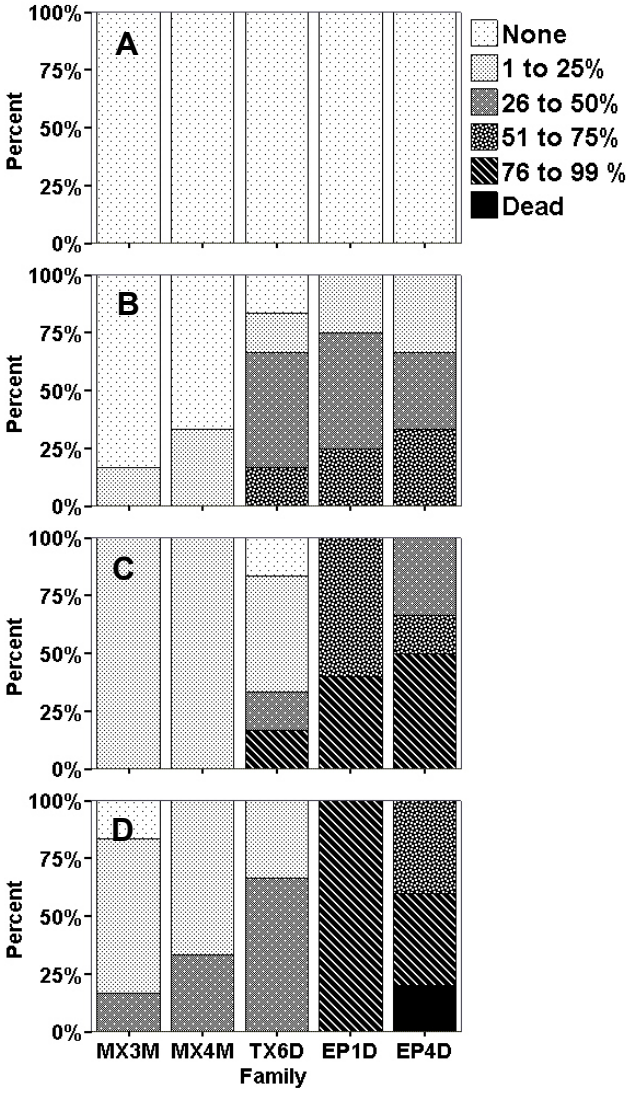

Figure 23. Foliar damage ratings of 5 open-pollinated families of *Taxodium distichum* at four levels of foliar spray salinity. The four levels of foliar salts were 0 $mg \cdot L^{-1}$ (A), 800 $mg \cdot L^{-1}$(B), 1260 $mg \cdot L^{-1}$(C), 1850 $mg \cdot L^{-1}$(D). The various shading of portions of each bar represents the percentage of individuals in a family at each salinity level receiving a certain rating. n = 6.

Taxodium is known to show foliar damage when exposed to salt spray, which decreases its value in the landscape (Arnold, 2002). Visual appearance of landscape plant material is crucial to its successful usage (Devitt et al., 2003). Although only the foliar damage ratings differed among families in short term exposure, this study is still useful for screening material for foliar applied salt tolerance because of the importance of aesthetics in landscape horticulture and urban forestry. Devitt et al. (2003) claim that their screening, which was based on a visual dieback rating, was valid although they observed no effect on growth or plant water relations.

When selecting material for sites with soil salinity issues it is important to select genotypes that have been demonstrated to tolerate these conditions. Most genotypes will likely be suitable for low or even moderate levels of soil salts, but at high soil salinities the tolerance appears to be highly genotype dependent, rather than having a strong geographic pattern. However, in most landscape situations, foliar exposure to salts is more limiting for *Taxodium* than soil-borne salts. Foliar salt tolerance may be driven by leaf surface characteristics. It may be desirable to select genotypes that limit the amount of water that remains on the leaf and the duration of the exposure. The more western provenances seem to have appropriate leaf surface characteristics. The faster growth rate of the Mexican genotypes has the added benefit of raising the canopy level above the wetting zone of overhead sprinkler systems more rapidly. In areas were the planting of these provenances is not limited by cold, the Mexican provenances are preferable to more eastern populations if foliar exposure to poor quality irrigation is expected.

CHAPTER VII

EVALUATION OF FIELD PERFORMANCE OF SELECTED PROVENANCES OF *TAXODIUM*

Drought, salinity, and alkaline soils are common problems faced by many arborists, urban foresters, landscapers and homeowners. Kelsey and Hootman (1990) found that many urban street tree planter soils could be classified as saline or sodic, soil types that usually occur in arid or semi-arid areas with a higher evapotranspiration than precipitation. In many parts of the United States, drought and irrigation restrictions are becoming more common (Beeson et al., 2004).

Arnold (2002) defines ecotypic variation as "a distinct morphological or physiological form, or population, resulting from (natural) selection by a distinct ecological condition". It is the entire basis for provenance studies (Arnold, 2002). Zobel and Talbert (1984) define a provenance as "the original geographic area from which seed or other propagules were obtained" and equate it to the concepts of geographic source and geographic race. They also present a more useful definition of the concept as "a subdivision of a species consisting of genetically similar individuals, related by common descent, and occupying a particular territory to which it has become adapted through natural selection" (Zobel and Talbert, 1984). Provenances can be determined by numerous features of the geographic origin, including latitude, altitude, precipitation, temperature, soil, and day length (Zobel and Talbert, 1984). The identification of adapted provenances allows industry professionals to more closely

tailor their plant selections to specific situations and can offer “the largest, cheapest and fastest gains” in tree improvement programs seeking an improved product for use in difficult ecophysiographic situations (Zobel and Talbert, 1984).

Taxodium distichum (L.) Rich. is a widely adaptable tree species for landscape use, tolerating both wet and dry soils, and air pollution (Cox and Leslie, 1988; Wasowski and Wasowski, 1997). Watson (1983) reports tolerance of varying nutrient availability conditions, a wide range of soil aeration conditions, and somewhat extreme pH levels. It is fast growing, has reliable feathery foliage, and a nice form (Arnold, 2002; Cox and Leslie, 1988). Two varieties, var. *distichum* (baldcypress) and var. *imbricarium* (Nutt.) Croom (pondcypress), have fairly good fall color some years, while var. *mexicana* Gordon (Montezuma cypress) remains semi-evergreen (Arnold, 2002). It is an extremely long-lived tree, with a life span of up to 700 years possible (Cox and Leslie, 1988). All of these factors allow *Taxodium* to tolerate many environmental stresses, making this a promising choice for urban landscapes. However, there are a few limitations to this species. While it is tolerant of substantial soil salts, it tends to defoliate when leaves come into contact with salty irrigation water, tends to develop chlorosis on sites with high pH, and has a tendency to "brown out" in periods of extended or severe drought (Arnold, 2002).

The purpose of this study is to evaluate provenances of *Taxodium* in an effort to select those which yield individuals that are most vigorous and adaptable to a range of environmental conditions.

Materials and Methods

Open-pollinated family identity was coded with four alphanumeric characters. The first two letters signify the general geographic origin of the mother tree. 'MX' signifies south Texas and Mexico, 'TX' signifies central Texas, and 'EP' denotes the southeastern U.S. The numeral is unique to an open-pollinated family from a given geographic area. The final letter indicates the taxonomic variety. 'M' indicates that the open-pollinated family belongs to the variety *mexicanum*, 'D' indicates var. *distichum*, and 'I' indicates var. *imbricarium*.

Open-pollinated families of *Taxodium distichum* were collected in the late summer and fall of 2003. Seeds from a single mother tree at several locations (Table 17, Fig. 24) representing the ecophysiographic variation throughout the species' range were collected and stratified (90 d at 2 ºC). Localities representing "normal" seed sources (mesic, acidic eastern U.S. sites), as well as sites representing more extreme environmental conditions (more xeric, alkaline western U.S. and Mexican sites) were sampled. Seeds were planted on 12 Mar. 2004, and germination took place in Mar. and Apr. of 2004. On 8-10 Apr. 2004, seedlings were transplanted into 2.5 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with 3 pine bark : 1 coarse perlite (by volume) mix amended with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Company, Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ 0N-0P-0K-6Ca-3Mg-12S-17Fe micronutrient fertilizer (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), and 4.15 $kg \cdot m^{-3}$ $CaMgCO_3$ (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown under 55% light

exclusion in a nursery area and irrigated as needed. Plants were trained as needed to a single leader and no additional fertilizer was applied.

Table 17. Localities of mother trees providing seeds of open-pollinated families of *Taxodium distichum* collected in the late summer and fall of 2003 used in field trials.

Family[z]	**Latitude**	**Longitude**	**Locale**	**pH**[y]
MX1M	25º52′48″N	97º27′0″W	Southmost, TX	7.8
MX2M	25º18′36″N	104º38′24″W	Rio Nazas, MX	na
MX3M*	19º30′0″N	98º54′36″W	Bolleros, MX	na
MX4M	27º51′0″N	101º7′48″W	Rio Sabinas, MX	na
MX5M	26º4′12″N	97º54′36″W	Progreso, TX	7.8
TX1D*	29º55′12″N	98º48′0″W	Guadalupe River, TX	na
TX2D	30º4′12″N	99º17′24″W	Guadalupe River, TX	7.2
TX3D*	29º47′24″N	99º35′24″W	Sabinal River, TX	na
TX4D*	29º43′12″N	99º45′0″W	Frio River, TX	na
TX5D*	29º9′36″N	99º28′12″W	Sabinal River, TX	na
TX6D	29º0′36″N	98º34′48″W	Atascosa River, TX	7.1
TX7D*	29º46′12″N	98º8′24″W	Guadalupe River, TX	na
TX8D	29º52′48″N	97º55′48″W	San Marcos River, TX	7.5
EP1D	32º20′24″N	94º42′0″W	Lake Cherokee, TX	7.2
EP2D	29º48′0″N	91º47′24″W	Iberia Parish, LA	na
EP3D*	29º5′24″N	91º12′6″W	Bayou Teche, LA	na
EP4D	30º36′0″N	87º54′36″W	Mobile Bay, AL	5.2
EP5I	30º27′0″N	88º6′36″W	Fowl River, AL	4.8
EP6D	30º24′36″N	88º54′0″W	Biloxi, MS	6.2
EP7D*	30º23′24″N	88º55′48″W	Biloxi, MS	na
EP8D	31º33′36′ N	91º26′24″W	Mississippi River, LA	7.0
EP9D*	30º7′12″N	93º43′48″W	Sabine River, TX	na

[z] Families followed by * were only evaluated at the College Station site.
[y] Soil samples were collected where possible to determine soil pH at the collection site, na = not available.

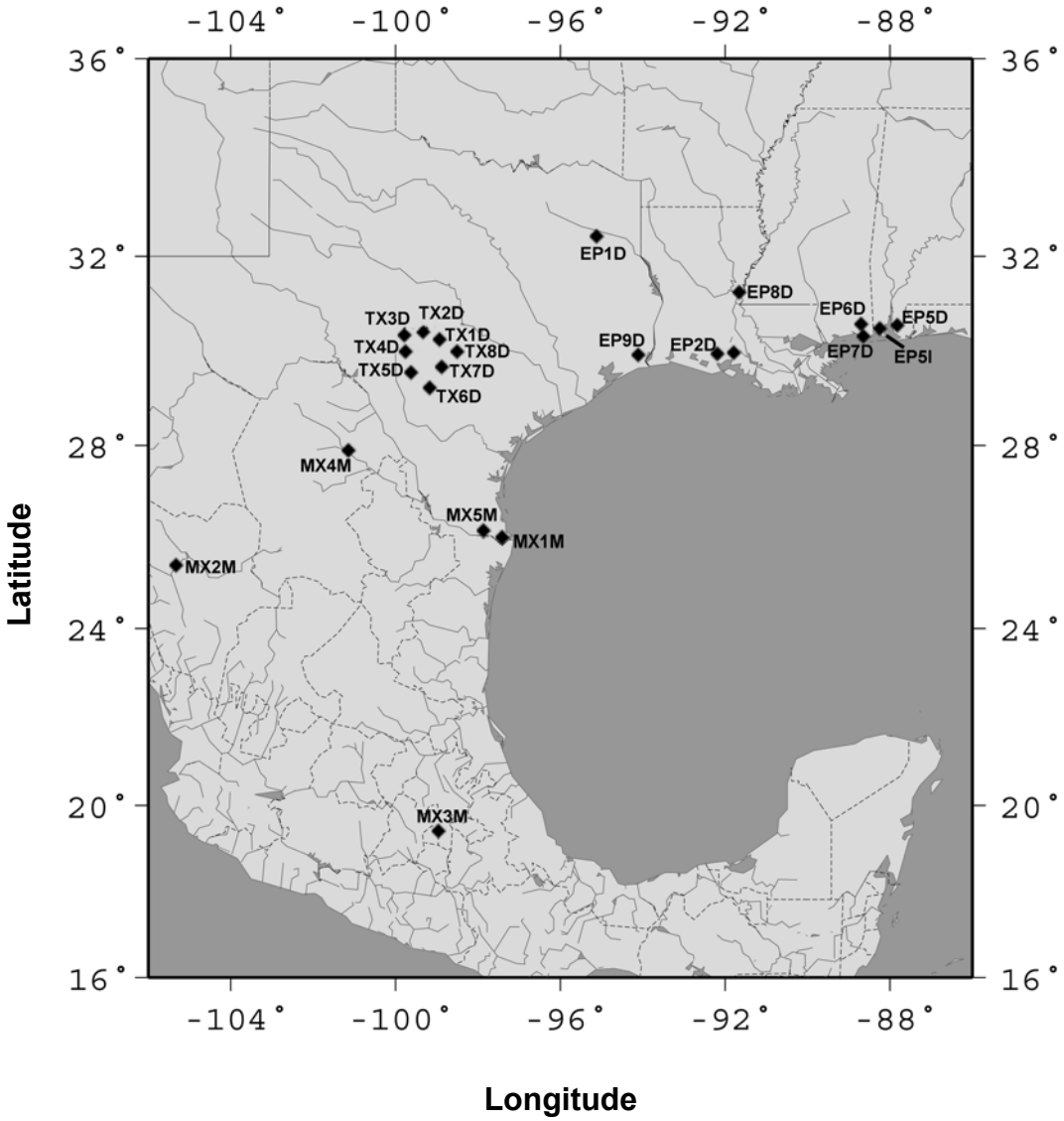

Figure 24. Locations of mother trees providing seeds for open-pollinated families of *Taxodium distichum* used in field trials. Family identities are indicated beside the symbols.

Evaluation took place at three locations. The first field site was located at the Texas A&M Research and Extension Center at Dallas (borderline USDA hardiness zone 8a/7b). The soil at the site is an Austin silty clay, 1 to 3 % slopes and has a pH of 8.0±0.5. The trees were irrigated as needed throughout the study and no additional fertilizer was added. Seedlings were planted on 18 June 2004. Plants were arranged in a randomized complete block design with 13 families in 20 blocks containing two replications of each family per block. Tree heights and trunk diameters were measured at the time of planting and again in the next three Decembers. The second field site was located at the Texas A&M Research and Extension Center at Overton (USDA hardiness zone 8a). The soil at the site is a Bowie very fine sandy loam, 1 to 4 % slopes and has a pH of approximately 6.5. The trees were irrigated as needed the first year only. Seedlings were planted on 29 June 2004. Plants were arranged in a randomized complete block design with 13 families in 20 blocks containing 2 replications of each family per block. Tree heights and trunk diameters were measured at the time of planting and again in the next three Decembers. The third field site was located at the Texas A&M University Horticulture Farm (USDA hardiness zone 8b). The soil at the site is a Tabor fine sandy loam, 0 to 2 percent slopes and has a pH of approximately 7.0. The trees were irrigated as needed and no additional fertilizer was added. Seedlings were planted on 23 July 2004. Plants were arranged in a randomized complete block design with 22 families in 20 blocks containing 2 replications of each family per block. Tree heights and trunk diameters were measured at the time of planting and again in the next three Decembers.

Growth indices for both height and trunk diameter were calculated as follows: growth index = (new measure – previous measure)/previous measure. This is analogous to relative growth rate calculations, except it is based on non-destructive measures rather than dry masses (Arnold et al., 2007). Data for each site was analyzed separately. Growth data were analyzed using univariate analysis in the GLM procedure and hierarchical cluster analysis in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, IL.). Survival data were analyzed with the Chi-square procedure in SPSS.

Results and Discussion

Each of the three field sites presented different environmental conditions. The Dallas site was the most alkaline and was irrigated as needed. The Overton site had acidic soils (pH 6.5±0.4) and was irrigated only the first season. It received below normal precipitation the following two seasons (Fig. 25). The College Station site was the least adverse site, receiving irrigation as needed and having a soil pH of 7.0±0.2. At all three sites, the time by family interaction was significant ($P \leq 0.05$) and none of the parameters measured were significantly affected by block ($P \geq 0.05$).

Overton

There was significant variation in tree survival in the second and third growing season at the Overton field site (Fig. 26). The Chi-square test for survival in both seasons for open-pollinated family was highly significant ($P \leq 0.0001$). In both years, the western genotypes generally had higher survival percentages than genotypes from more mesic, eastern sources (Fig. 26). This pattern is especially striking in the third season (2006) cumulative survival where none of the eastern families had above 25%

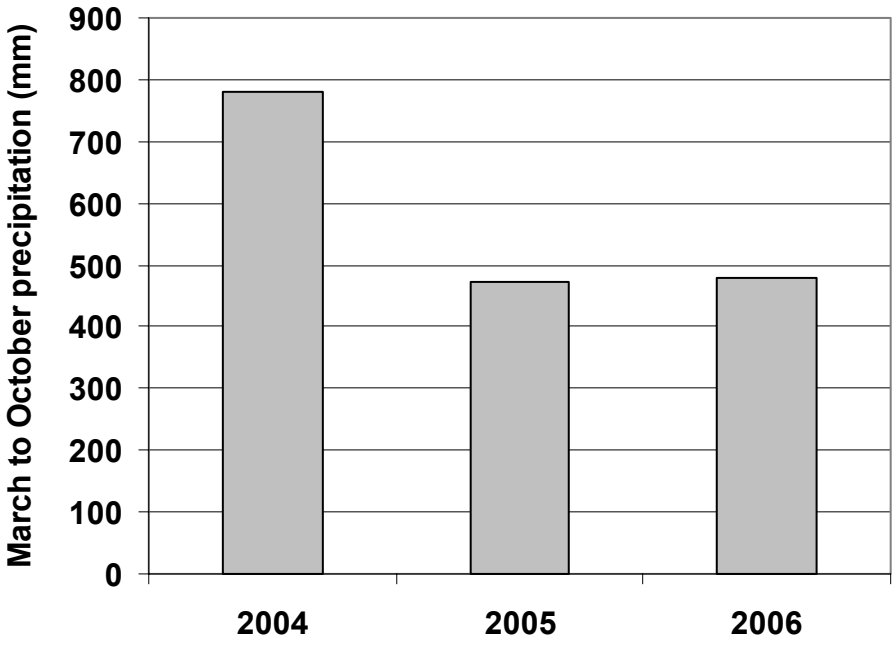

Figure 25. Observed precipitation during the growing season in 2004, 2005, and 2006 in Overton, Texas. Irrigation was also provided as needed in 2004, but not 2005 or 2006.

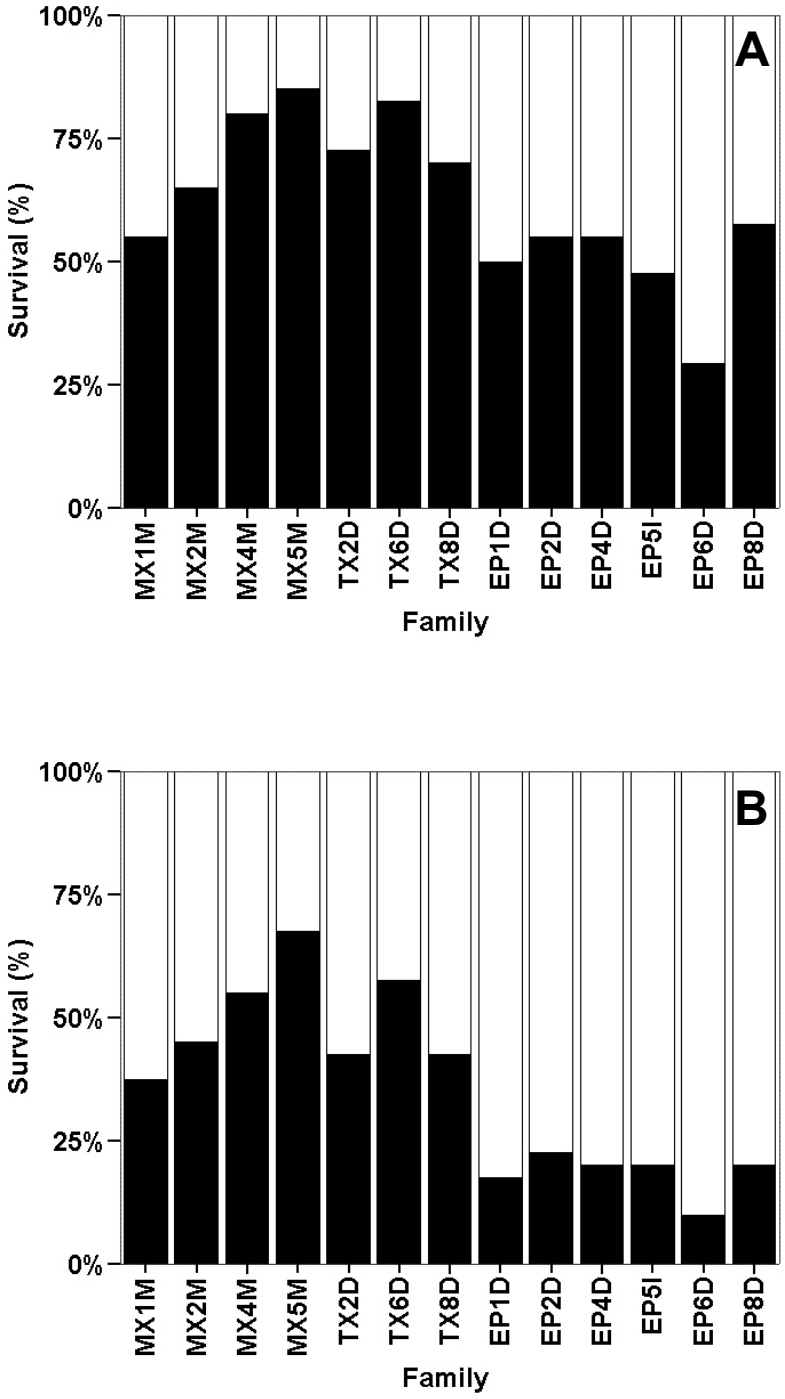

Figure 26. Percent cumulative survival of 13 open-pollinated families of *Taxodium distichum* for 2005 (A) and 2006 (B) in Overton, Texas.

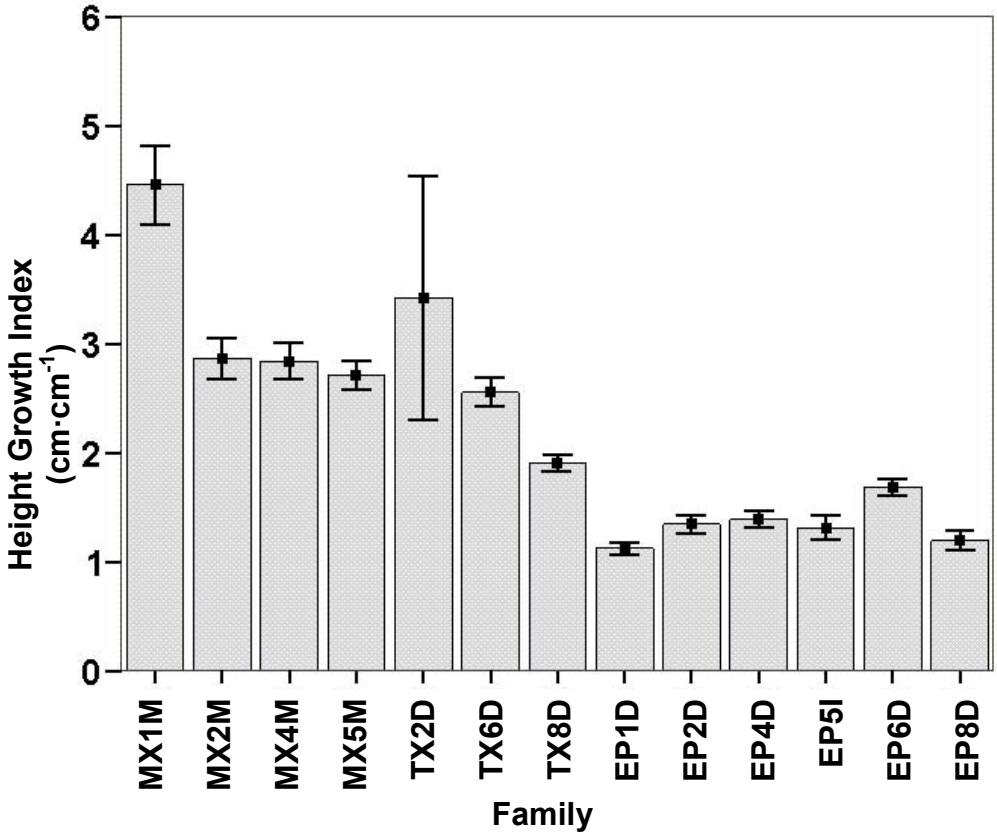

Figure 27. Height growth index for 13 open-pollinated families of *Taxodium distichum* in 2005 at Overton, Texas. Symbols represent means ± standard error of 40 observations.

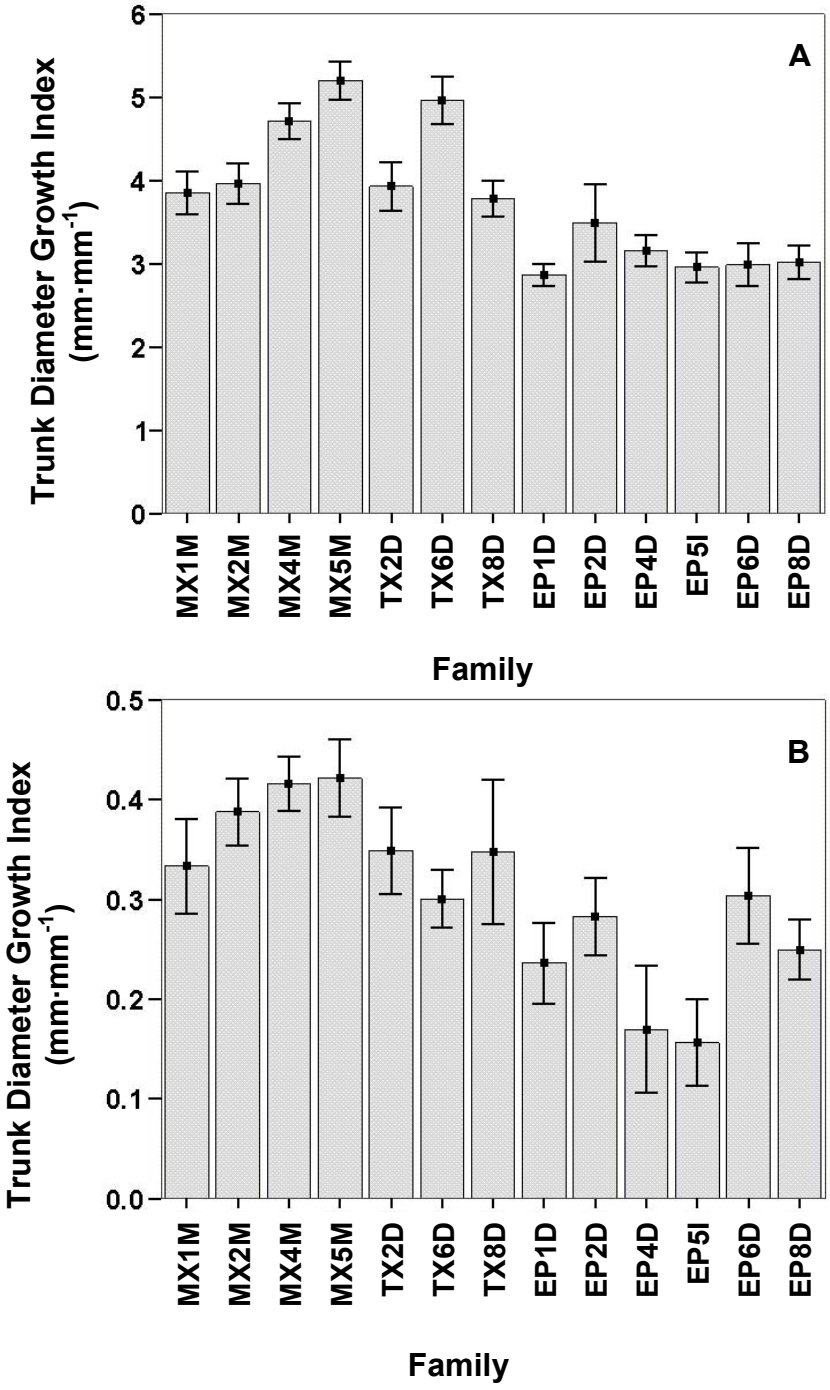

Figure 28. Trunk diameter growth index for 13 open-pollinated families of *Taxodium distichum* in 2005 (A) and 2006 (B) at Overton, Texas. Symbols represent means ± standard error of 40 observations.

cumulative survival (Fig 26 b). There was significant variation in the height growth index (Fig. 27) and the trunk diameter growth index for 2005 among families ($P \leq 0.0001$) (Fig. 28). In 2006, only the variation in trunk diameter growth indices was significant ($P \leq 0.0001$). A similar pattern to that observed in the survival percentages was evident. The western families grew faster in height (Fig. 27) and trunk diameter during 2005 and in trunk diameter during 2006, when compared to eastern genotypes (Fig. 28).

This pattern is similar to that observed in the greenhouse-based drought screenings discussed in chapter IV. These results support the conclusions of the greenhouse-based studies that western populations of *Taxodium distichum* are generally more drought tolerant than eastern populations. Field performance under xeric conditions improved as populations were sampled from east to west in the U.S. and then south into Mexico, following a general environmental gradient of decreasing precipitaion. This pattern was observed by Li (1998) in *Eucalyptus* L'Her. provenances and St. Hilaire and Graves (2001) in *Acer saccharum* Marsh. seedlings. Shoemake et al. (2004) report that in *Platanus occidentalis* L., genotypes from xeric regions performed better than those from mesic areas when grown on a xeric site. The implication is that when choosing *Taxodium* for use in more xeric conditions, care should be taken to select western genotypes.

Dallas

There was significant ($P \leq 0.0001$) variation in both growth indices for all three years at the Dallas site (Fig. 29). Height growth indices for the western genotypes were, again, generally higher compared to those of eastern populations (Fig. 29). The same pattern was observed in trunk diameter growth indices of open-pollinated families (Fig. 30). The final heights and trunk diameters were also significantly different among open-pollinated families ($P \leq 0.0001$). The faster growth rate of the western genotypes is reflected by the larger final sizes (Fig. 31, Fig. 32).

As discussed in chapter V, the western populations were also less chlorotic. Presumably, the western genotypes are the best adapted to the alkaline soils present at the site, which translates into the trees from these families growing faster and being "greener". Marcar et al. (2002) reported a similar trend in high pH tolerance of *Eucalyptus camaldulensis* Dehnh. provenances in Australia. They report that seedlings from localities with higher soil pH showed less growth reduction when grown in substrates with a pH of 9.5. Wood et al. (1998) found a similar geographic pattern for Zn deficiency in pecan [*Carya illinonensis* (Wangenh.) K.Koch] provenances. They found that Texas and Mexican provenances tended to cluster together, separate from sources north and east of Texas. Taxonomic ranking also seems to correlate with performance on this alkaline site. This is to be expected, as varietal status in this species has a strong geographic component (Denny and Arnold, 2007). Genotypes from south Texas and Mexico all belong to var. *mexicana*, montezuma cypress.

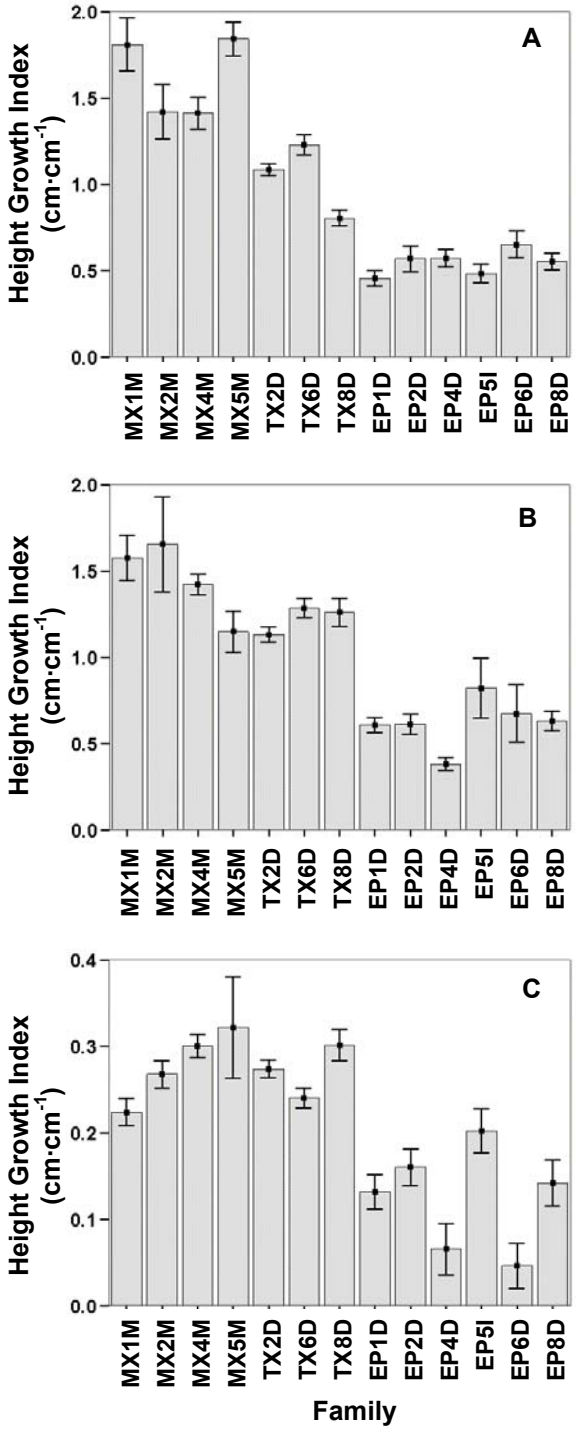

Figure 29. Height growth index for 13 open-pollinated families of *Taxodium distichum* in 2004 (A), 2005 (B), and 2006 (C) at Dallas, Texas. Symbols represent means ± standard error of 40 observations.

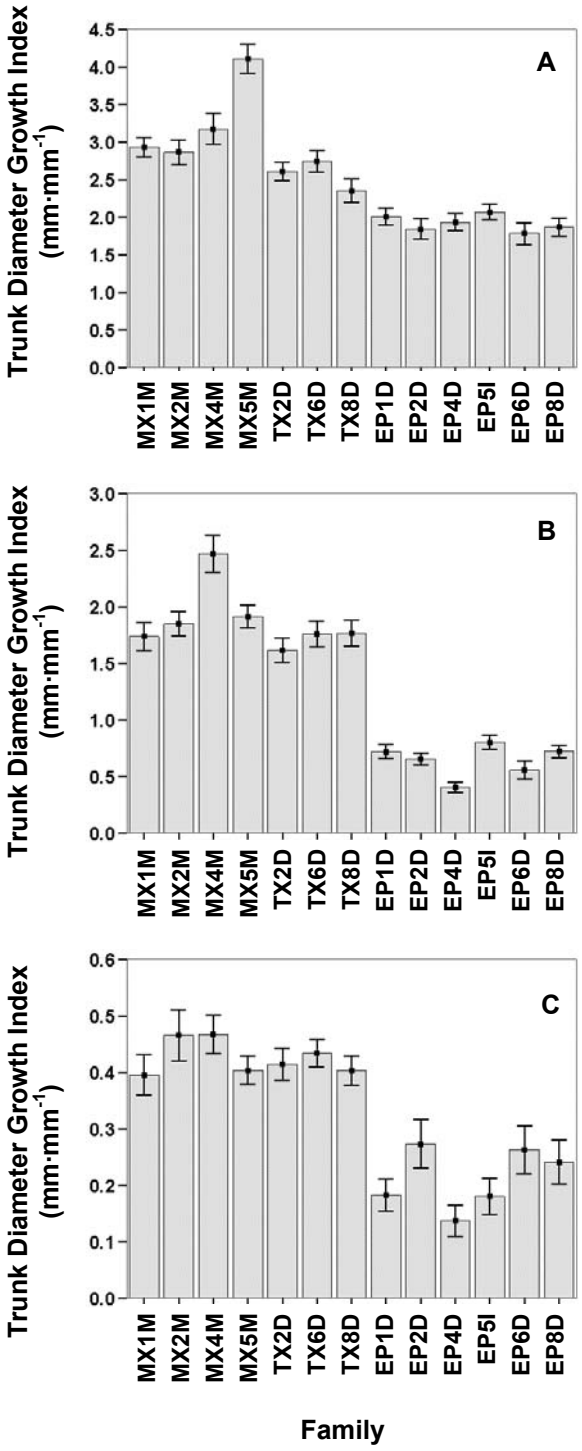

Figure 30. Trunk diameter growth index for 13 open-pollinated families of *Taxodium distichum* in 2004 (A), 2005 (B), and 2006 (C) at Dallas, Texas. Symbols represent means ± standard error of 40 observations.

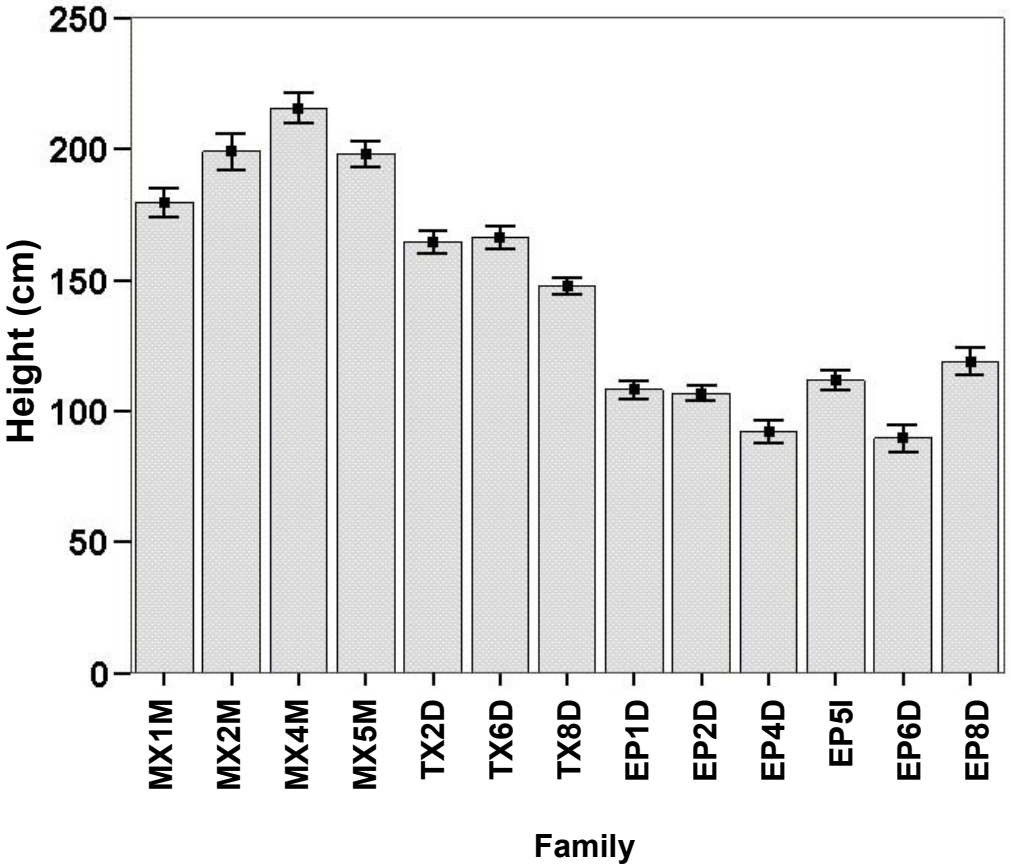

Figure 31. Tree height for 13 open-pollinated families of *Taxodium distichum* in 2006 at Dallas, Texas. Symbols represent means ± standard error of 40 observations.

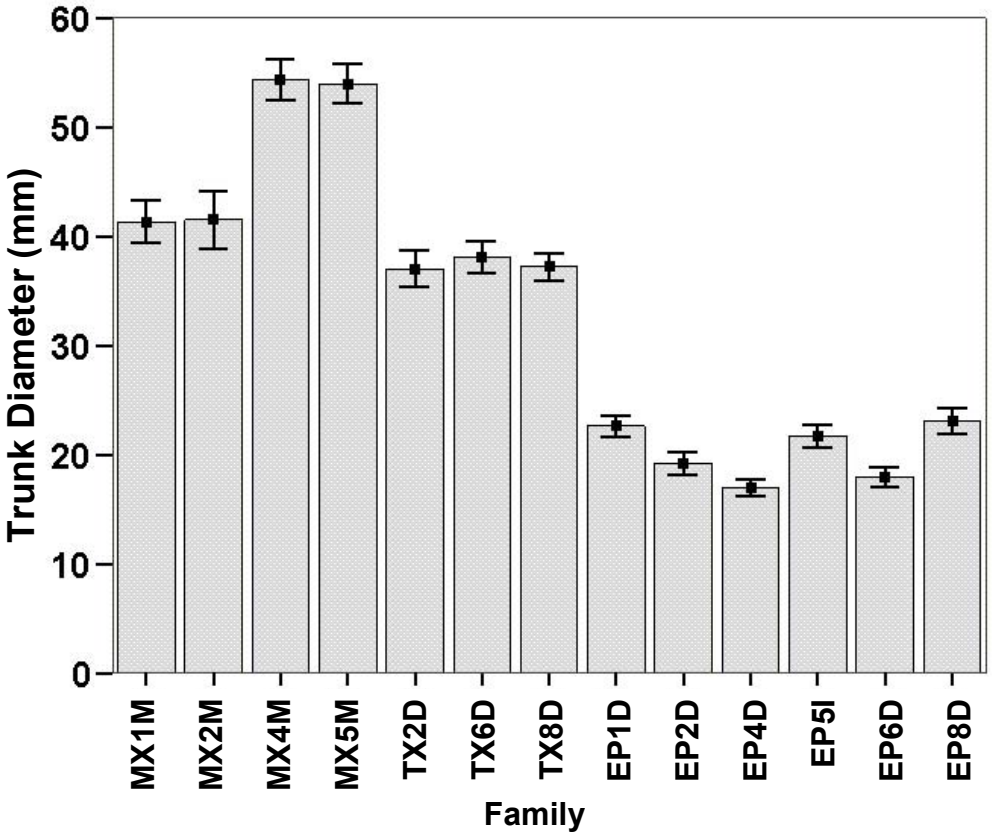

Figure 32. Trunk diameter for 13 open-pollinated families of *Taxodium distichum* in 2006 at Dallas, Texas. Symbols represent means ± standard error of 40 observations.

All of the genotypes in central Texas are var. *distichum*, baldcypress. The vast majority of the sampled eastern genotypes are also var. *distichum*, the exception being family EP5I from the Fowl River in Alabama. This family was var. *imbricarium*, pondcypress. Montezuma cypress seems to be more tolerant of an alkaline site than the other varieties. If this variety is excluded, and var. *distichum* is considered alone, there is still a strong geographic component to the variation in tolerance of alkaline soils. There are numerous examples of variation in alkalinity tolerance among related taxa. Ben-Ya'acov and Michelson (1995) report racial differences among *Persea americana* Mill. rootstocks in resistance to "lime-induced chlorosis", Shi and Byrne (1995) report on variation in bicarbonate tolerance of *Prunus* L. rootstocks and Valdez-Aguilar and Reed (2006) found differential alkalinity tolerance in two cultivars of *Hibiscus rosa-sinensis* L.

College Station

Growth varied among open-pollinated families at the College Station site. The height growth indices of the trees differed among open-pollinated families in 2004 ($P \leq 0.0001$) and 2005 ($P \leq 0.0001$), however no significant difference was observed in 2006 ($P \geq 0.05$) (data not shown). With the exception of families TX3D and TX5D, trees from central Texas and South Texas and Mexico generally grew in height more rapidly than eastern genotypes (Fig. 33). The trunk diameter growth indices of the trees differed among open-pollinated families in 2004 ($P \leq 0.0001$) and 2005 ($P \leq 0.01$) (Fig. 34); however, no significant difference was observed in 2006 ($P \geq 0.05$) (data not shown). With the exception of family MX2M, trees from South Texas and Mexico, grew in trunk

diameter more rapidly than central Texas and eastern genotypes in 2004 (Fig. 34). Family TX4D (from central Texas) grew as rapidly in 2004 as Mexican and south Texas genotypes (Fig. 34). In 2005 differences in trunk diameter growth were much less pronounced (Fig. 34). Families MX4M and MX5M (Mexican and south Texas genotypes) grew the fastest, while families EP5I and EP8D were the poorest performers in trunk growth (Fig. 34). Open-pollinated families varied in both final tree height ($P \leq 0.0001$) (Fig. 35) and final trunk diameter ($P \leq 0.0001$) (Fig. 36). The genotypes from south Texas and Mexico, with the exception of family MX3M, tended to be taller compared to families from both central Texas and the eastern U.S. (Fig. 35). The central Texas and eastern U.S. genotypes had very similar final heights (Fig. 35). A geographic pattern was less evident in the differences in final trunk diameter among open-pollinated families, however the two families with the largest mean trunk diameters were both south Texas (MX5M) and Mexican (MX4M) genotypes (Fig. 36).

The differences observed in growth of the open-pollinated families at the other sites were less pronounced at the College Station location. This may be due to the less extreme soil and moisture conditions experienced by the trees. Taxonomic ranking seems to correlate with field performance on this site as well. The genotypes belonging to var. *mexicana* (montezuma cypress) generally grew more rapidly compared to the var. *distichum* (baldcypress) and var. *imbricarium* (pondcypress) genotypes. Arnold (2002) reports that montezuma cypress grows more quickly than the other two varieties of *Taxodium distichum*.

When selecting plant material for an alkaline or xeric site, Mexican and south Texas genotypes should be preferred, followed by central Texas genotypes. On less extreme sites, preference should still be given to genotypes from Mexico and south Texas, because of their more rapid growth rates. However, the probable greater cold hardiness of genotypes from central Texas based on their more northern latitudes of origin may dictate their use on alkaline sites in colder regions.

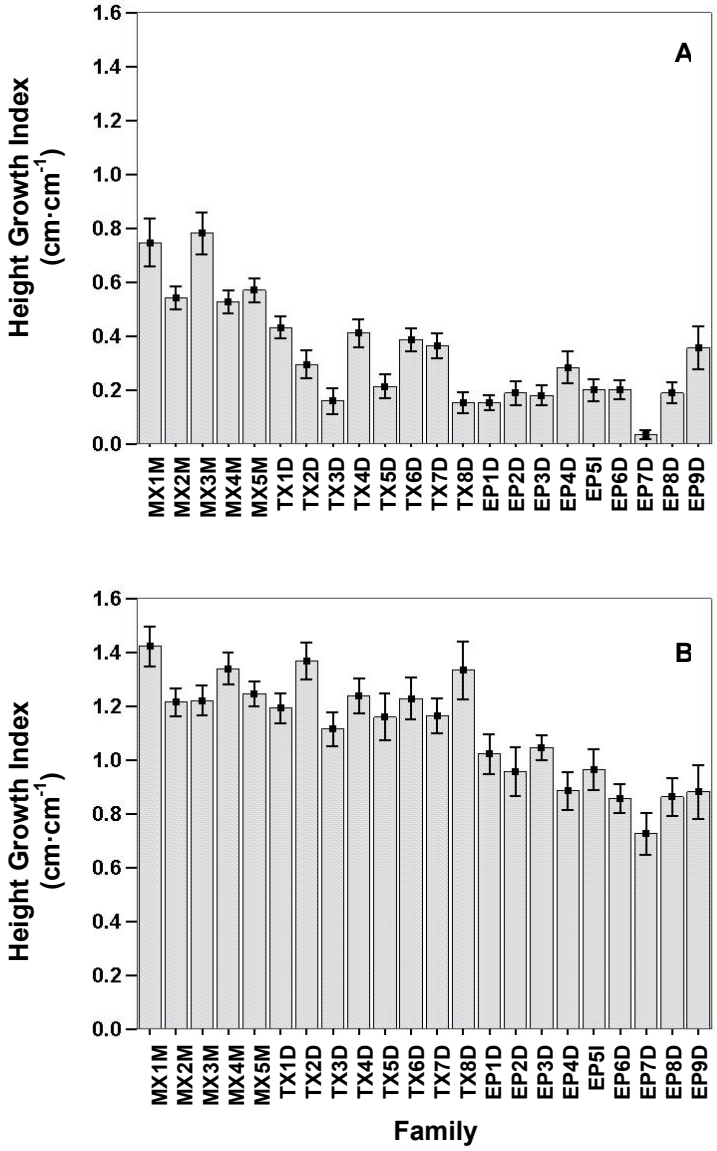

Figure 33. Height growth index for 22 open-pollinated families of *Taxodium distichum* in 2004 (A) and 2005 (B) at College Station, Texas. Symbols represent means ± standard error of 40 observations.

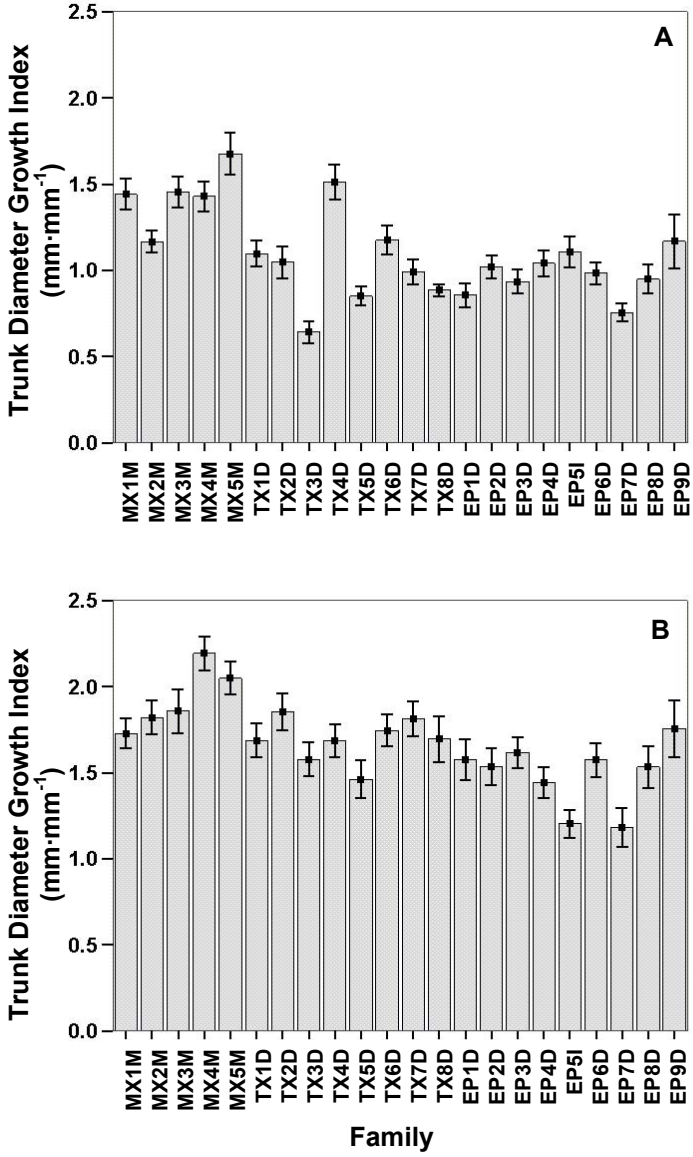

Figure 34. Trunk diameter growth index for 22 open-pollinated families of *Taxodium distichum* in 2004 (A) and 2005 (B) at College Station, Texas. Symbols represent means ± standard error of 40 observations.

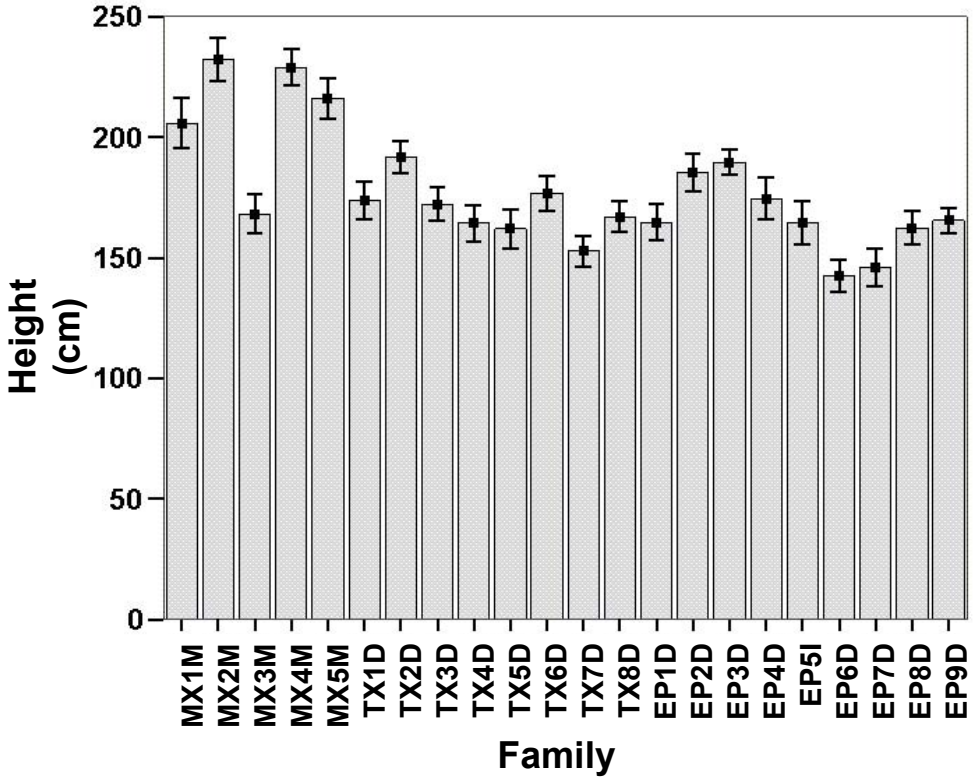

Figure 35. Tree height for 22 open-pollinated families of *Taxodium distichum* in 2006 at College Station, Texas. Symbols represent means ± standard error of 40 observations.

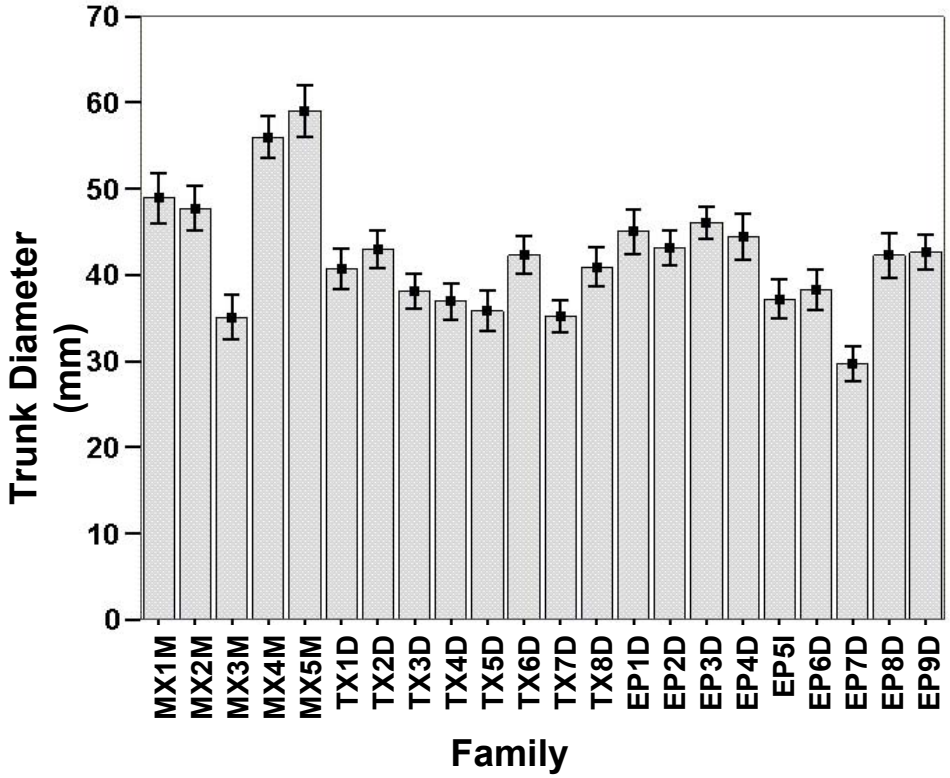

Figure 36. Trunk diameter for 22 open-pollinated families of *Taxodium distichum* in 2006 at College Station, Texas. Symbols represent means ± standard error of 40 observations.

CHAPTER VIII

SUMMARY

Taxodium distichum (L.) Rich. is a widely adaptable tree species for landscape use, tolerating both wet and dry soils, and air pollution (Cox and Leslie, 1988; Wasowski and Wasowski, 1997). Watson (1983) reports tolerance of varying nutrient availability conditions, a wide range of soil aeration levels, and somewhat extreme pH levels. It is fast growing, has reliable feathery foliage, and a nice form (Arnold, 2002; Cox and Leslie, 1988). Two varieties, var. *distichum* (baldcypress) and var. *imbricarium* (Nutt.) Croom (pondcypress), have fairly good fall color some years, while var. *mexicana* Gordon (Montezuma cypress) remains semi-evergreen (Arnold, 2002). It is an extremely long-lived tree, with a life span of up to 700 years possible (Cox and Leslie, 1988). All of these factors allow *T. distichum* to tolerate many environmental stresses, making this a promising choice for urban landscapes. However, there are a few limitations to this species. While it is tolerant of substantial soil salts, it tends to defoliate when leaves come into contact with salty irrigation water, tends to develop chlorosis on sites with high pH, and has a tendency to "brown out" in periods of extended or severe drought (Arnold, 2002).

Arnold (2002) defines ecotypic variation as “a distinct morphological or physiological form, or population, resulting from (natural) selection by a distinct ecological condition”. It is the entire basis for provenance studies (Arnold, 2002). Zobel and Talbert (1984) define a provenance as “a subdivision of a species consisting of genetically similar individuals, related by common descent, and occupying a

particular territory to which it has become adapted through natural selection". The identification of adapted provenances allows industry professionals to more closely tailor their plant selections to specific situations and can offer "the largest, cheapest and fastest gains" in tree improvement programs seeking an improved product for use in difficult ecophysiographic situations (Zobel and Talbert, 1984). In forest management, the concept of seed source and provenance are widely recognized and used (Zobel and Talbert, 1984). However, in landscape horticulture and urban forestry it is less widely utilized (Arnold, 1995 and 2002).

As interest in issues such as seed source, provenance, genetic pollution and threatened and endangered plant species grows, so does the need for an understanding of the relatedness and evolutionary history of plants. Appropriate taxonomy and nomenclature become much more important. *Taxodium* (L.) Rich. is a genus of landscape trees, included in many plant materials courses across the country. It has been treated variously in the horticulture literature as having one, two, or three species (Denny and Arnold, 2007). The most appropriate treatment is one species with three botanical varieties. The appropriate name for baldcypress is *Taxodium distichum* (L.) Rich. var. *distichum*. The appropriate name for pondcypress is *T. distichum* var. *imbricarium* (Nutt.) Croom. The appropriate name for montezuma cypress is *T. distichum* var. *mexicanum* Gordon.

Taxodium distichum var. *mexicanum*, montezuma cypress, is a valuable ornamental tree species tolerant of a wide range of cultural conditions. However, little is know about the propagation requirements of this species. A study was conducted 1) to

determine the effect of previously recommended seed treatments for baldcypress (*T. d.* var. *distichum*) or pondcypress (*T. d.* var. *imbricarium*) on montezuma cypress seeds, and 2) to determine the effects of stratification in combination with pre-germination treatments on germination of montezuma cypress seeds. Open-pollinated seeds were collected from a single tree in Southmost, Texas. Seven pre-germination treatments and three stratification periods were applied to the seeds. The study revealed that if immediate germination of ripe seed is desired, then the best treatments are the citric acid soak and the hot water baths, however, if seeds can be stratified, then no pre-germination seed treatment is needed. Citric acid scarification and hot water baths produced the best germination. Stratification hastened germination rates and cumulative mean germination percentages. Stratification for 45 d appears to be sufficient, although for the best pre-germination treatments stratification requirements were less pronounced.

Screening studies of open-pollinated families for drought tolerance in a greenhouse suggest a geographic component to variation in drought tolerance of *Taxodium distichum*. The observed geographic pattern is what might be expected. The open-pollinated families from mesic eastern localities were less tolerant of drought than open-pollinated families from more xeric western populations. Further drought screenings suggest that *T. distichum* likely relies on both drought avoidance and drought tolerance strategies to deal with drought stress. It seems to avoid drought by limiting water loss from the shoots rather than increasing biomass partitioning to the roots, while the drought tolerance component seems to take the form of osmotic adjustment. Variation in these traits seems to show the same geographic pattern described above,

with the occurrence of these important traits increasing as populations are sampled from east to west in the U.S. and then south into Mexico, following a general environmental gradient of decreasing precipitation. The implication is that when choosing *Taxodium* for use in more xeric conditions, care should be taken to select western genotypes.

A three year field study provided a reliable screening technique for alkalinity tolerance. It clearly shows that there is a geographic component to alkalinity tolerance in *Taxodium distichum*, with genotypes from Mexico and south Texas showing the most tolerance, followed by central Texas genotypes, then open-pollinated families from the eastern U.S. Taxonomic placement also seems to correlate with performance on an alkaline site. This is to be expected, as varietal status in this species has a strong geographic component (Denny and Arnold, 2007). Montezuma cypress seems to be more tolerant of an alkaline site than the other varieties. If this variety is excluded, and var. *distichum* is considered alone, there is still a strong geographic component to the variation in tolerance of alkaline soils. When selecting plant material for an alkaline site, genotypes from Mexico and south Texas should be preferred, followed by central Texas genotypes.

All of the open-pollinated families screened were relatively tolerant of low levels of substrate salinities. Niknam and McComb (2000) state that at moderate levels of salinity, salt exclusion is the main adaptive strategy and tolerance of high ion concentrations is important at high levels of salinity. There is also the osmotic component to salinity stress to be considered. The increased tolerance of selected open-pollinated families at higher salinity levels was likely due to a combination of both

osmotic and ionic stress tolerance. The increased osmotic stress avoidance was observed in the less negative pre-dawn xylem water potentials of the more tolerant families at moderate and high levels of salinity. Relatively greater tolerance to water stress for some of the more tolerant families had been observed in the drought tolerance screenings of open-pollinated families, but not for all. This may imply that there is a different stress tolerance mechanism for salinity stress than those stress tolerance mechanisms utilized to cope with drought stress, perhaps improved ion exclusion capacity.

In general, open-pollinated families from Mexico were more tolerant of salt spray than families from the southeastern U.S. The family from central Texas was intermediate in tolerance. Although there seems to be a geographic pattern to the tolerance differences observed, the difference may be due to the differential leaf wetting times. No data was taken on amount of time the leaves remained wet, but it was observed that the Mexican families tended to be harder to wet and dried the fastest, followed by a family from central Texas, then families from the southeastern U.S. This difference may be attributable to leaf surface differences. The more western provenances are waxier in appearance and may have a thicker cuticle. This would explain the response pattern observed in this study. The poor performance of a family from Mobile Bay in Alabama was somewhat surprising because of its origin. The mother tree was growing in close proximity to the beach. This family was suspected to have increased tolerance to foliar salts due to its natural exposure to salt spray. It did show a slight improvement over the other "eastern-type" family, but not in comparison to more western provenances.

When selecting material for sites with soil salinity issues it is important to select genotypes that have been demonstrated to tolerate these conditions. Most genotypes will likely be suitable for low or even moderate levels of soil salts, but at high soil salinities the tolerance appears to be highly genotype dependent, rather than having a strong geographic pattern. However, in most landscape situations, foliar exposure to salts is more limiting for *Taxodium* than soil-borne salts. Foliar salt tolerance appears to be driven by leaf surface characteristics. Care should be taken to select genotypes that limit the amount of water that remains on the leaf and the duration of the exposure. The more western provenances seem to have appropriate leaf surface characteristics. The faster growth rate of the Mexican genotypes has the added benefit of raising the canopy level above the wetting zone of overhead sprinkler systems more rapidly. In areas were the planting of these provenances is not limited by cold, the Mexican provenances are preferable to more eastern populations if foliar exposure to poor quality irrigation is expected.

Drought response in the field is similar to that observed in the greenhouse-based drought screenings discussed in Chapter III. These results support the conclusions of the greenhouse-based studies that western populations of *Taxodium distichum* are generally more drought tolerant than eastern populations. Field performance under xeric conditions improved as populations were sampled from east to west in the U.S. and then south into Mexico, following a general environmental gradient of decreasing precipitation.

Field evaluations demonstrated that genotypes belonging to var. *mexicanum* (montezuma cypress) generally grew more rapidly compared to the var. *distichum* (baldcypress) and var. *imbricarium* (pondcypress) genotypes. These evaluations also suggest that when selecting plant material for an alkaline or xeric site, south Texas and Mexican genotypes should be preferred, followed by central Texas genotypes. On less extreme sites, preference should still be given to genotypes from Mexico and south Texas, because of their more rapid growth rates. However, the probable greater cold hardiness of genotypes from central Texas based on their more northern latitudes of origin may dictate their use on alkaline sites in colder regions.

Further research is needed to fully evaluate the level of tolerance of the best performing genotypes to verify the findings of the screenings. Clonal materials from trees selected for superior performance should also be further evaluated in replicated field trials. Absolute cold hardiness of superior genotypes needs to be determined, especially for south Texas and Mexican genotypes, so that proper seed source or clonal recommendations can be made. Foliar salt tolerance should evaluated more thoroughly so that potential tolerance mechanisms can be identified, allowing selection of genotypes with traits that confer greater tolerance.

LITERATURE CITED

Abrams, M.D., M.E. Kubiske, and K.C. Steiner. 1990. Drought adaptations and responses in five genotypes of *Fraxinus pennsylvanica* Marsh.: photosynthesis, water relations and leaf morphology. Tree Physiol. 6:305-315.

Aiton, W. 1789. Hortus kewensis. George Nicol, London.

Allen, J.A., J.L. Chambers, and D. McKinney. 1994 Intraspecific variation in the response of *Taxodium distichum* seedlings to salinity. For. Ecol. and Mgmt. 70:203-214.

Allen, J.A., S.R. Pezeshki, and J.L. Chambers. 1996. Interaction of flooding and salinity stress on baldcypress (*Taxodium distichum*). Tree Physiology 16:307-313.

Amtmann, A. and D. Sanders. 1999. Mechanisms of Na^+ uptake by plant cells. pp. 76-112. In: Advances in botanical research, vol. 29. Academic Press, New York.

Arnold, M.A. 1995. Enthusiasm for 'excellence'. American Nurseryman 181(6):12-13.

Arnold, M.A. 2002. Landscape plants for Texas and environs, sec. ed. Stipes Publ. L.L.C., Champaign, Ill. p. 1088.

Anold, M.A., G.V. McDonald, D.L. Bryan, G.C. Denny, W.T. Watson, and L. Lombardini. 2007. Below-grade planting adversely affects survival and growth of tree species from five different families. Arboriculture and Urban Forestry 33(1):64-69.

Beeson, Jr., R.C., M.A. Arnold, T.E. Bilderback, B. Bolusky, S. Chandler, H.M. Gramling, J.D. Lea-Cox, J.R. Harris, P.J. Klinger, H.M. Mathers, J.M. Ruter, and T.H. Yeager. 2004. Strategic vision of container nursery irrigation in the next ten years. J. Environ. Hort. 22:113-115.

Beilman, A.P. 1947. What happens when all kinds of southern cypress are grown in the north? Southern Lumberman 174 (2179):48.

Ben-Ya'acov, A. and E. Michelson. 1995. Avocado rootstocks. pp. 381-429. In: J. Janick (ed.). Horticultural reviews, volume 17. John Wiley & Sons, Inc., Indianapolis, Ind.

Bresnan, D.F., W.A. Geyer and G. Rink. 1996. Variation among green ash of differing geographic origins outplanted in Kansas. J. Arboriculture 22:113-116.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought – from genes to the whole plant. Functional Plant Biology 30:239-264.

Conner, W.H. and L.W. Inabinette. 2005. Identification of salt tolerant baldcypress (*Taxodium distichum* (L.) Rich.) for planting in coastal areas. New Forests 29:305-312.

Cox, P.W. and P. Leslie. 1988. Texas trees: a friendly guide. Corona Publ. Co., San Antonio, Texas.

Cregg, B.M. 1994. Carbon allocation, gas exchange, and needle morphology of *Pinus ponderosa* genotypes known to differ in growth and survival under imposed drought. Tree Physiology 14:883-898.

Creech, D. and Y. Yunlong. 2003. *Taxodium* x 'Nanjing Beauty': A new landscape plant for the south. HortScience 38:1292-1293 (Abstract).

Croom, H.B. 1837. Cat. Pl. New Bern, N.C.

Correll, D.S. and M.C. Johnston. 1970. Manual of the vascular plants of Texas. Texas Research Foundation, Renner, Texas. pp. 75-76.

Denny, G.C. and M.A. Arnold. 2007a. Taxonomy and nomenclature of baldcypress, pondcypress and montezuma cypress: one, two or three species? HortTechnology 17:125-127.

Denny, G.C. and M.A. Arnold. 2007b. Pre-germination seed treatments and stratification affect germination of montezuma cypress. J. Environ. Hort. 25:1-3.

Devitt, D.A., R.L. Morris, and D.S. Neuman. 2003. Impact of water treatment on foliar damage of landscape trees sprinkle irrigated with reuse water. J. Environ. Hort. 21:82-88.

Fan, S., T.J. Blake, and E. Blumwald. 1994. The relative contribution of elastic and osmotic adjustments to turgor maintenance of woody species. Physiologia Plantarum 90:408-413.

Faulkner, S. and J. Toliver. 1983. Genetic variation of cones, seeds, and nursery-grown seedlings of baldcypress (*Taxodium distichum* (L.) Rich.) provenances. pp.281-288. In: Southern Forest Tree Improvement Committee (Eds.), Proc. of the 17th Southern For. Tree Improvement Conf., June 6-9, Univ. of Georgia, Athens, Ga.

Flint, H.L. 1974. Geographically based physiological variation in *Taxodium distichum* (L.) Rich. HortScience 9:301 (abstract).

Giridhar, J., B. Pai, and W.R. Graves. 1995. Seed source affects seedling development and nitrogen fixation of *Maackia amurensis*. J. Environ. Hort. 13:142-146.

Greuter, W., J. McNeill, F.R. Barrie, H.M. Burdet, V. Demoulin, T.S. Filgueiras, D.H. Nicolson, P.C. Silva, J.E. Skog, P. Trehane, N.J. Turland, D.L. Hawksworth (eds.). 2000. International code of botanical nomenclature (St Louis Code). Regnum Vegetabile 138. Koeltz Scientific Books, Königstein, Germany.

Griffiths, M. 1994. The new Royal Horitcultural Society dictionary: Index of garden plants. Timber Press, Portland, Ore.

Griffin, J.J., T.G. Ranney, and D.M. Pharr. 2004. Heat and drought influence photosynthesis, water relations, and soluble carbohydrates of two ecotypes of redbud (*Cercis canadensis*). J. Amer. Soc. Hort. Sci. 129:497-502.

Gucci, R., G. Aronne, L. Lombardini, and M. Tattini. 1997. Salinity tolerance in *Phillyrea* species. New Phytologist 135:227-234.

Harborne, J.B. 1998. Phytochemical methods. A guide to modern techniques of plant analysis, third ed. Chapman & Hall, London. p. 302

Jacoby, B. 1999. Mechanisms involved in salt tolerance of plants. pp. 97-124. In: M. Pessarakli (ed.). Handbook of plant and crop stress. Marcel Decker Inc., New York.

Jones, M.M., N.C. Turner, and C.B. Osmond. 1981. Mechanisms of drought resistance. p. 15. In: Paleg, L.G. and D. Aspinall (eds.). Physiology and biochemistry of drought resistance in plants. Academic Press, Sydney, Austrailia.

Jull, L.G., T.G. Ranney, and F.A. Blazich. 1999. Heat tolerance of selected provenances of Atlantic white cedar. J. Amer. Soc. Hort. Sci. 124:492-497.

Kelsey, P. and R. Hootman. 1990. Soil resource evaluation for a group of sidewalk street tree planters. J. Arboric. 16:113-117.

Kramer, P.J. 1983. Water relations of plants. Academic Press. New York. pp. 342-475.

Krauss, K.W., J.L. Chambers, and J.A. Allen. 1996. Intraspecific variation in physiological response of baldcypress (*Taxodium distichum* (L.) Rich.) to a rapid influx of saltwater. pp.183-189. In: Flynn, K.M. (Ed.), Proc. of the Southern Forested Wetlands Ecol. and Mgmt. Conf., March 25-27. Clemson, S.C.

Krauss, K.W., J.L. Chambers, and J.A. Allen. 1998. Salinity effects and differential germination of several half-sib families of baldcypress from different seed sources. New Forests 15:53-68.

Krauss, K.W., J.L. Chambers, J.A. Allen, B.P. Luse, and A.S. DeBosier. 1999. Root and shoot responses of *Taxodium distichum* seedlings subjected to saline flooding. Environ. Expt. Bot. 41:15-23.

Krüssmann, G. [H. Warda (ed.), M. Epp (translator)]. 1985. Manual of cultivated conifers. Timber Press, Portland, Ore..

Lambers, H., F.S. Chapin III, and T.L. Pons. 1998. Plant physiological ecology. Springer-Verlag New York, Inc., New York. pp. 154-204, 239-292.

Levitt, J. 1980. Responses of plants to environmental stresses. Volume II: Water, radiation, salt and other stresses, 2nd ed. Academic Press, New York. pp. 25-210, 365-454.

Li, C. 1998. Some aspects of leaf water relations in four provenances of *Eucalyptus microtheca* seedlings. For. Ecol. and Mgmt. 111:303-308.

Li, C., F. Berninger, J. Koskela, and E. Sonninen. 2000. Drought response of *Eucalyptus microtheca* provenances depend on seasonality of rainfall in their place of origin. Australian J. of Plant Physiol. 27:231-238.

Liberty Hyde Bailey Hortorium. 1976. Hortus third: A concise dictionary of plants cultivated in the United States and Canada. Macmillan, New York.

Lickey, E.B. and G.L. Walker. 2002. Population genetic structure of baldcypress (*Taxodium distichum* [L.] Rich. var. *distichum*) and pondcypress (*T. distichum* var. *imbricarium* [Nuttall] Croom): Biogeographic and taxonomic implications. Southeastern Naturalist 1:131-148.

Lickey, E.B., F.D. Watson, and G.L. Walker. 2002. Differences in bark thickness among populations of baldcypress [*Taxodium distichum* (L.) Rich. var. *distichum*] and pondcypress [*T. distichum* var. *imbricarium* (Nuttall) Croom]. Castanea 67:33-41.

Linnaeus, C. 1753. Species plantarum. Impensis Laurentii Salvii, Stockholm, Sweden.

Marcar, N.E., Y. Zohar, J. Guo, and D.F. Crawford. 2002. Effect of NaCl and high pH on seedling growth of 15 *Eucalyptus camaldulensis* Dehnh. provenances. New Forests 23:193-206.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, San Diego, Calif.

McMillan, C. 1974. Differentiation in habitat response in *Taxodium distichum, Taxodium mucronatum, Platanus occidentalis,* and *Liquidambar styraciflua* from the United States and Mexico. Vegetatio 29:1-10.

Merchant, A. and M. Adams. 2005. Stable osmotica in *Eucalyptus spathulata* – responses to salt and water deficit stress. Functional Plant Biol. 32:797-780.

Merchant, A., M. Tausz, S.K. Arndt, and M.A. Adams. 2006. Cyclitols and carbohydrates in leaves and roots of 13 *Eucalyptus* species suggest contrasting physiological responses to water deficit. Plant, Cell and Environ. 29:2017-2029.

Middleton B.L. and K.L. McKee. 2004. Use of a latitudinal gradient in bald cypress (*Taxodium distichum*) production to examine physiological controls of biotic boundaries and potential responses to environmental change. Global Ecol. Biogeography 13:247-258.

Missouri Botanical Garden, 2006. VAST nomenclatural database and associated authority files, 12 June 2006, <http://mobot.mobot.org/W3T/Search/vast.html>.

Murphy, J.B. and R.G. Stanley. 1975. Increased germination rates of baldcypress and pondcypress seed following treatments affecting the seed coat. Physiol. Plant. 35:135-139.

Miyamoto, S., I. Martinez, M. Padilla, A. Portillo, and D. Ornelas. 2004. Landscape plant lists for salt tolerance assessment. Texas A&M Univ., Agric. Res. Ctr. at El Paso, Texas. p. 1.

Murphy, J.B. and R.G. Stanley. 1975. Increased germination rates of baldcypress and pondcypress seed following treatments affecting the seed coat. Physiol. Plant. 35:135-139.

Navari-Izzo, F. and N. Rascio. 1999. Plant response to water-deficit conditions. pp. 231-270. In: M. Pessarakli (ed.). Handbook of plant and crop stress. Marcel Decker. Inc., New York.

Neufeld, H.S. and R.O. Teskey. 1986. Variation in the amount of foliage on woody shoots and its effects on water relations parameters derived from pressure-volume curves. Can. J. For. Res. 16:239-243.

Niknam, S.R. and J. McComb. 2000. Salt tolerance screening of selected Australian woody species – a review. For. Ecol. and Management 139:1-19.

Nuttall, T. 1818. The genera of North American plants. Vol. 2: 224. Facsimile of 1818 edition. Hafner Publishing Co., New York. 1971.

Pai, J.G.B. and W.R. Graves. 1995. Seed source affects seedling development and nitrogen fixation of *Maackia amurensis*. J. Environ. Hort. 13:142-146.

Pezeshki, S.R., R.D. DeLaune, and H.S. Choi. 1995. Gas exchange and growth of baldcypress seedlings from selected U.S. Gulf Coast populations: responses to elevated salinities. Can. J. For. Res. 25:1409-1415.

Pezeshki, S.R., R.D. DeLaune, and W.H. Patrick, Jr. 1998. Effect of salinity on leaf ionic content and photosynthesis of *Taxodium distichum* L. American Midland Naturalist 119:185-192.

Pugnaire, F.I., L. Serrano and J. Pardos. 1999. Constraints by water stress on plant growth. pp. 271-283. In: M. Pessarakli (ed.). Handbook of plant and crop stress. Marcel Decker. Inc., New York.

Richard, L.C.M. 1810. Note sur les plantes dites conifers. Annales du muséum national d'histoire naturelle 16: 296-299.

Richards, L.A. (ed). 1969. Diagnosis and improvement of saline and alkali soils. USDA handbook 60, U.S. Dept. of Agric., Washington, D.C.

Roberts, S.W., B.R. Strain, and K.R. Knoerr. 1981. Seasonal variation of leaf elasticity in four forest tree species. Physiologia Plantarum 52:245-250.

Schrader, J.A. and W.R Graves. 2000. Seed germination and seedling growth of *Alnus maritima* from its three disjunct populations. J. Amer. Soc. Hort. Sci. 125:128-134.

Schopmeyer, C.S. 1974. Seeds of Woody Plants in the United States: USDA Handbook No. 450. Forest Service, USDA, Washington, D.C.

Sharma, G.K. and L. Madsen. 1978. Variation in baldcypress from different habitats. J. Tenn. Acad. Sci. 53:115-116.

Shi, Y. and D.H. Byrne. 1995. Tolerance of *Prunus* rootstocks to potassium carbonate-induced chlorosis. J. Amer. Soc. Hort. Sci. 120:283-285.

Shoemake, L.J., M.A. Arnold, and F.T. Davies, Jr. 2004. Provenance impacts transplant establishment and adventitious root regeneration of sycamore. J. Amer. Soc. Hort. Sci. 129:360-367.

Shoemake, L.J. 1996. Effects of half-sib family selection on the root regeneration potential and initial landscape establishment of container-grown sycamore, *Platanus occidentalis* L. M.S. Thesis. Texas A&M Univ., College Station, Texas.

Shoemake, L.J. and M.A. Arnold. 1997. Half-sib family selection improves container nursery and landscape performance of Sycamore. J. Environ. Hort. 15:126-130.

St. Hilaire, R. 2001. Seed coat treatments influence germination of *Taxodium mucronatum*. Desert Plants 17(1):15-18.

St. Hilaire, R. and W.R. Graves. 2001. Stability of provenance differences during development of hard maple seedlings irrigated at two frequencies. HortScience 36:654-657.

Taiz, L. and E. Zeiger. 1998. Plant physiology, sec. ed. Sinauer Associates, Inc., Publishers, Sunderland, Mass.

The International Plant Names Index. 2006. The International Plant Names Index, 12 June 2006, <http://www.ipni.org/ipni/plantsearch?request_type=search&output_format=query&>.

Tsumura, Y., N. Tomaru, Y. Suyama, and S. Bacchus. 1999. Genetic diversity and differentiation of *Taxodium* in the south-eastern United States using cleaved amplifies polymorphic sequences. Heredity 83:229-238.

Tuomela, K. 1997. Leaf water relations in six provenances of *Eucalyptus microtheca*: a greenhouse experiment. For. Ecol. and Mgmt. 92:1-10.

Turner, B.L., H. Nichols, G.C. Denny, and O.Doron. 2003. Atlas of the vascular plants of Texas. Sida, Botanical Miscellany. 24. V.2., Botanical Research Institute of Texas, Fort Worth, Texas.

Turner, N.C. 1988. Measurement of plant water status by the pressure chamber technique. Irrigation Sci. 9:289-308.

Turner, Jr., R.G. and E. Watson (eds.). 1999. Botanica. Barnes & Noble, Inc., New York.

United States Department of Agriculture – Agricultural Research Service. 2006. USDA National Plant Germplasm System, 12 June 2006, <http://www.arsgrin.gov/npgs/searchgrin.html>.

Valdez-Aguilar, L.A. and D.W. Reed. 2006. Comparison of growth and alkalinity-induced responses in two cultivars of hibiscus (*Hibiscus rosa-sinensis* L.). HortScience 41:1704-1708.

Vines, R.A. 1960. Trees, shrubs and woody vines of the Southwest: A guide for the states of Arkansas, Louisiana, New Mexico, Oklahoma, and Texas. Univ. of Texas Press, Austin.

Wahome, P.K., H.H. Jesch, and I. Grittner. 2001. Mechanisms of salt tolerance in two rose rootstocks: *Rosa chinensis* 'Major' and *R. rubiginosa*. Scientia Hort. 87:207-216.

Wasowski, S. and A. Wasowski. 1997. Native Texas plants: landscaping region by region. Gulf Publishing Co., Houston, Texas.

Watson, F.D. 1983. A taxonomic study of pondcypress and baldcypress. Ph.D. Dissertation. North Carolina State University, Raleigh, N.C. p. 198.

Watson, F.D. 1993. *Taxodium* Richard. pp. 403-404. *In*: Flora of North America Editorial Committee (eds.). Flora of North America North of Mexico. Oxford Univ. Press, New York.

White, R.H., M.C. Engelke, S.J. Anderson, B.A. Ruemmele, K.B. Marcum, and G.R. Taylor, II. 2001. Zoysiagrass water relations. Crop Science 41:133-138.

Wood, B.W., L.J. Grauke, and J.A. Payne. 1998. Provenance variation in pecan. J. Amer. Soc. Hort. Sci. 123:1023-1028.

Zobel, B. and J. Talbert. 1984. Applied forest tree improvement. Waveland Press, Inc., Prospect Heights, Ill. pp. 58-116.

Zwack, J.A. and W.R. Graves. 1998. Leaf water relations and plant development of three freeman maple cultivars subjected to drought. J. Amer. Soc. Hort. Sci. 123:371-375.

Zwack, J.A., W.R. Graves and A. M. Townsend. 1999. Variation among red and freeman maples in response to drought and flooding. HortScience 34:664-668.

VITA

Name: Geoffrey Carlile Denny

Address: 144 Lisa St., Canutillo, TX 79835

Email Address: gcdenny@tamu.edu

Education: B.S., Horticulture, Texas A&M University, 2000
M.A., Plant Biology, The University of Texas at Austin, 2002